LIGHT DAMAGE AND PHOTORECEPTOR RENEWAL

By

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LIGHT DAMAGE AND PHOTORECEPTOR RENEWAL

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Major Department: Neuroscience

This study investigated the effects of four levels of cyclic light (12 hours light, 12 hours dark) on retinas of albino rats raised from birth under low intensity cyclic light (6 lux). The objectives were (1) to determine the threshold light intensity which produces retinal damage, (2) to determine what effects low intensity cyclic light has on pigment epithelial function and (3) to determine whether the rate of photoreceptor outer segment renewal is affected by light conditions which produce either mild or moderate levels of retinal damage. The extent of light damage to the retinal tissue was determined from direct examination of the tissue at the light microscopic level and electrophysiological recordings of the b-wave component of the electroretinogram (ERG). Histology was evaluated using both morphometric and semi-quantitative rating techniques.

The effects of light on pigment epithelial function were determined through d.c. recordings of the c-wave component of the ERG and sodium azide-induced changes in the eye's resting potential. Photoreceptor renewal was assessed by measuring protein synthesis using light microscopic autoradiographic techniques.

Light intensities of 65 or 130 lux produced small but significant changes in retinal morphology and responsiveness; however, these changes were not consistently observed throughout the test period and therefore may be considered transient. Intensities of 270 and 1345 lux produced permanent retinal degeneration in fewer than 7 days. The threshold light intensity was determined to be approximately 1.0 log unit above the rearing light condition.

C-wave and azide-induced responses recorded from albino rats raised under 6 lux were significantly smaller than those recorded from retinas of dark-reared albino rats. These results suggest that albino rat pigment epithelium is functionally impaired by light intensities below those which damage the neural retina.

The rate of photoreceptor outer segment renewal in the retinas of albino rats raised under 6 lux was shown to be unaffected after exposure to 130 or 270 lux for 1, 3 or 7 days. These results, while indicating that renewal processes continue during toxic light exposure, suggest that photoreceptor cells do not adjust their renewal rate under conditions which produce cellular damage.

CHAPTER 1
INTRODUCTION

In 1966 Noell and his associates reported the damaging effects of ordinary light upon the retina of albino rats (Noell et al., 1966). These effects were described electrophysiologically as reductions in the amplitudes of the a- and b-waves of the electroretinogram (ERG) which accompanied degeneration of photoreceptor cells and the retinal pigment epithelium. It is now well established that the retina can be damaged by exposure to visible light. Damage can occur as a result of either a brief exposure to high intensity light (Ham et al., 1976; Kuwabara, 1970; Noell et al., 1966) or prolonged exposures to relatively low intensity light (LaVail, 1976; O'Steen and Lytle, 1971; O'Steen et al., 1972; Parnavelas and Globus, 1976; Rapp and Williams, 1980; Shear et al., 1973) and is more readily produced in albino than in pigmented animals (Noell et al., 1966; Rapp and Williams, 1980).

The principal morphological change which occurs in the retina as a result of exposure to toxic light is the degeneration and eventual death of the photoreceptor cells (Noell et al., 1976; O'Steen and Lytle, 1971; Shear et al., 1973). The results of several studies suggest that the outer segment of the photoreceptor cell, which is the light sensitive part of the cell, is the primary site of retinal damage (Grignolo et al., 1969; O'Steen and Lytle, 1971; Shear et al., 1973) and that retinal degeneration induced by light exposure is dependent on the amount of light absorbed by the visual pigment in the outer segments of the photoreceptor cells (Kaitz and Auerbach, 1979; Noell, 1979; Noell et al., 1966). The photoreceptor outer segment is comprised of a number of membranous discs which are stacked in an orderly array within the outer segment plasma membrane. Following light exposure, outer segment disc membranes become disrupted, showing separation and formation of tubules and vesicles. During the final stages of degeneration, the damaged membranes are removed from the retina by phagocytic cells of vascular origin, pigment epithelial cells and Muller cells (Hansson, 1970; O'Steen and Lytle, 1971; O'Steen and Karcioglu, 1974). The pigment epithelium may also be damaged under certain light and environmental conditions (Noell, 1980). Light-induced damage to the pigment epithelium is discussed in CHAPTER 4.

The ERG has been used extensively in light toxicity studies to measure functional integrity of the outer retina following light exposure (Cicerone, 1976; Gorn and Kuwabara, 1967; Noell and Albrecht, 1971; Noell et al., 1966; Rapp and Williams, 1979; Saavedra and Pellegrino de Iraldi, 1976). The ERG is comprised of four major components: (1) the a-wave, (2) the b-wave, (3) the c-wave and (4) a dc component (Brown, 1980). The amplitudes of the a- and b-waves of the ERG are most frequently used to evaluate function following light damage. The a-wave of the ERG results in part from a stimulus-induced change in the ionic fluxes or 'dark currents' which flow along and through photoreceptors in the absence of stimulation. Light stimulation reduces the permeability of the outer segment to cations, the consequences of which ultimately lead to a hyperpolarization of the photoreceptor cell. It is the hyperpolarization of photoreceptor cells which generates the negative portion of the a-wave of the ERG [for a review, see Brown, 1980]. The b-wave is generated by Müller [glial] cells within the retina in response to alterations in the ionic composition of extracellular fluid caused by activity in nearby nerve cells [Miller and Dowling, 1970]. Since the soma of the Muller cells lie within the inner nuclear layer, generation of the b-wave by the Muller cells is probably secondary to neuronal activity in the inner nuclear layer. Thus, the amplitude of the b-wave is often used as an indicator of inner nuclear layer activity and of outer retinal sensitivity.

The major changes which have been observed in the a- and b-wave components of the ERG in response to light damage are a reduction in the amplitudes of both waveforms and an increase in the latency and threshold of the b-wave [Gorn and Kuwabara, 1967; Noell et al., 1966; Rapp and Williams, 1979; Saavedra and Pellegrino de Iraldi, 1976]. The decline in the amplitudes of the a- and b-waves is intensity dependent and is related to retinal tissue damage [Gorn and

Kuwabara, 1967). In the case of the b-wave, the degeneration of the photoreceptor cells does not appear to affect the ability of the retina to produce normal responses, but instead reduces the input to the inner retina and Müller cells in proportion to lowered rhodopsin levels in the retina (Rapp and Williams, 1979). In cases of severe damage, characterized by extensive loss of photoreceptor cells, the ERG is not recordable (Kuwabara, 1970).

One hypothesis concerning the molecular mechanism underlying retinal light damage (Noell et al., 1966; Noell, 1980) which has recently received much attention is that photooxidation reactions initiated by the action of light on pigments within the retinal tissues are primarily responsible for the damaging effects of light on the retina. The process of photooxidation can be broken down into three general reactions. First, molecules in the tissue absorb light. Second, these molecules transfer their energy to an acceptor molecule, which in this case would be oxygen, to produce an electronically excited singlet oxygen ion. Finally, singlet oxygen reacts with polyunsaturated fatty acids in outer segment membranes to produce lipid peroxides (Delmelle, 1979; Halliwell, 1981). Peroxides and some of their degradation products are highly cytotoxic and cause extensive damage to membranes, including decreases in membrane resistance and fluidity and eventual loss of membrane integrity.

Recent reports have implicated lipid peroxidation reactions in light damage (Delmelle, 1979; Kagan et al., 1973; Organisciak et al., 1981; Rapp et al., 1982; Stone et al., 1979; Wiegand et al., 1982). Support for this hypothesis is derived primarily from studies which show that (1) toxic light exposure leads to a significant increase in the accumulation of lipid hydroperoxides (Kagan et al., 1973; Organisciak et al., 1981) (2) there is a significant loss of docosahexaenoic acid from photoreceptor outer segments exposed to toxic light (Wiegand et al., 1982) and (3) the retina contains a number of intrinsic mechanisms for controlling lipid peroxidation (Farnsworth and Dratz, 1976; Hall and Hall, 1975; Winkler and Giblin, 1983). If lipid peroxidation reactions are primarily involved in the degeneration of photoreceptor cells following toxic light exposure, then an appropriate sensitizer molecule must be present in the retina.

The results of several studies investigating the effectiveness of various wavelengths of light in generating light damage (action spectrum) in the rat suggest that light damage is mediated by photoreceptor visual pigments (Kaitz and Auerbach, 1979; Noell, 1979; Noell et al., 1966). The results of these studies show that the action spectra for the damaging effects of light closely matches the visual pigment absorption spectrum and suggest that retinal degeneration induced by light is dependent on the amount of light absorbed by photopigment in the retina. The identity of the sensitizer molecule is still unknown. Delmelle

[1979] and Noell [1980] have suggested that rhodopsin bleaching may lead to the uncovering of intermediates which may act as sensitizer molecules. In addition, Noell [1980] has proposed that this molecule would have an absorption spectrum within that of the visual pigment or extending into this region, which would explain the previous reports for the action spectrum of light damage [Kaitz and Auerbach, 1979; Noell, 1979; Noell et al., 1966].

The complexity of photobiological reactions and the specific biological complexities and unique features of retinal cells have so far impeded the analysis of the mechanisms underlying light damage. The effects of toxic light are not limited to the photoreceptor cells and often involve the pigment epithelium [Grignolo et al., 1969; Hansson, 1971; Kuwabara, 1970; Lawwill et al., 1977; Noell et al., 1966; Tso et al,, 1972]. Therefore, it would seem reasonable that several light-induced reactions may provoke the retinal degeneration which occurs in response to toxic light exposure,

The present study examines the effects of low intensity cyclic light on the retina of the albino rat. Very few studies have investigated the effects of cyclic light on the retina and those studies, for most part, have not examined these effects during the initial stages of light exposure or under carefully defined lighting conditions. Electrophysiological and anatomical techiques were employed in this study to answer the following three questions:

(1) What is the minimum light intensity which produces retinal damage to the retinas of albino rats raised from birth to 15 weeks of age under controlled low intensity lighting conditions (ambient cage illumination = 6±2 lux) with a photoperiod of 12 hours light, 12 hours dark (CHAPTER 3)?

(2) Does low intensity light affect the functioning of the albino rat retinal pigment epithelium (CHAPTER 4)?

(3) Is the rate of photoreceptor outer segment synthesis and renewal in the albino rat retina affected by exposure to intensities of light which produce mild to moderate levels of retinal damage (CHAPTER 5)?

CHAPTER 2
MATERIALS AND METHODS

2.1 General Materials and Methods

2.1.1 Experimental Animals

2.1.1.1 Animal strains. Albino rats (Sprague-Dawley strain) were the primary animals used in these experiments. This particular strain of rat was chosen because useful parametric studies of retinal light damage are available for this strain (Rapp and Williams, 1980). Pregnant albino rats were purchased from Zivic Miller Labs (Allison Park, PA). All albino rats used in these experiments were selected from the offspring of these rats. Litters were reduced to 8 pups (4 female + 4 male) soon after birth, and the offspring were weaned at 3 weeks of age. They were randomly assigned to experimental groups at 15-17 weeks of age. In addition to albino rats, one group of adult pigmented rats (hooded Long-Evans strain), purchased from Charles River Labs (Waltham, MA), was also used.

2.1.1.2 Housing and lighting conditions. All animals were housed in polycarbonate cages (48x27x20cm, LxWxH) with stainless steel wire lids, four to a cage, except in the case of pups, which were housed with their mothers until weaning. The bottoms of the cages were covered with wood chip bedding, and food (Purina Laboratory Chow pellets, #5001) and water were accessible through the wire lids. During exposure to experimental light conditions, a wire mesh grid was placed on top of the bedding to prevent burrowing, and food was placed inside the cages so that the illumination within the cages was as uniform as possible. The cages were placed in plywood boxes (122x61x61cm, LxWxH), four cages to a box. Each box was painted on the interior with flat white paint and was fitted with two 1.2m General Electric cool white fluorescent tubes (40 W; color temperature = 4500°K) which were covered with a clear plexiglass light diffuser. Fluorescent light sources were used because (1) their spectrum closely approximates daylight, (2) high light intensities can be generated with very little heat output and (3) their color temperature is relatively independent of fluctuations in line voltage. Light fixtures were placed above the cages so that the level of illumination within the cages did not vary in any direction by more than 20% of the mean illumination value. Light intensity was controlled using dimmer controls (Leviton Model 6676-277) and black cloth filters. The illumination 3cm above the floor of cage (rat eye position) was estimated by measuring the luminance of a MacBeth Illuminometer test plate (reflectance = 0.79) with an SEI exposure meter and adjusting for plate reflectance to yield illuminance in lux. A small ventilation fan was installed in each box which kept the temperature within each box at 23±2°C.

Albino rats were raised from birth under controlled low intensity lighting conditions (ambient cage illumination = 6±2 lux) with a photoperiod of 12 hours light, 12 hours dark (12:12 L:D) unless otherwise specified. This light level was chosen for the rearing and control conditions because it has been shown that 6 lux fails to produce damage in the retina of Sprague-Dawley rats after 16 days of constant light exposure (Rapp and Williams, 1980). The pigmented rats were raised in a commercial facility but upon arrival in our laboratory, were also housed under controlled lighting conditions (12:12 L:D; 6±2 lux ambient cage illumination).

2.1.2 Histological Procedures

2.1.2.1 Fixation of eyes. Most eyes were fixed using the following procedure. Animals were injected with an overdose of sodium pentobarbitol and were then perfused through the heart with heparinized saline followed by a mixture of 2.0% glutaraldehyde and 0.6% formaldehyde in 0.1M cacodylate buffer (pH 7.4) containing 0.05% calcium chloride. A suture was placed at the dorsal most aspect of each eye (for orientation) and the eyes were enucleated. A small slit was made in the globes at the ora serrata and the eyes were immersed in fresh fixative and refrigerated overnight (0-4°C). In a few cases, eyes were processed as above but without initial perfusion fixation. After the eyes were fixed, they were opened along the ora serrata using iris scissors and the lenses were removed from the eye cups. The eye cups were then bisected along the superior-inferior vertical axis and rinsed in 0.1M cacodylate buffer in preparation for dehydration.

2.1.2.2 Dehydration and embedding of eyes. Eyes were dehydrated in 2,2-dimethoxypropane (DMP) using a two step procedure. First, the eyes were covered with distilled water and an equal volume of DMP. Dehydration was initiated by adding one drop of 1N HCl to the mixture with gentle shaking. The vial containing the tissue was then allowed to sit at room temperature for 10 minutes. The solution was then decanted from the vial, the eyes were reimmersed in DMP, and dehydration was initiated as before. After an additional 10 minutes at room temperature, eyes were cleared two times in absolute ethanol.

Eyes were embedded in JB-4 embedding medium. Infiltration of specimens with this plastic was accomplished by placing them in a series of ethanolic JB-4 solutions which contained increasing proportions of JB-4. The ratios of JB-4 to absolute ethanol used were 1:1 2:1 and 100% JB-4. Infiltration was carried out at 0-4°C and eyes remained in each solution for approximately 12 hours. Following infiltration, the eyes were oriented in the embedding plastic so that longitudinal sectioning with respect to the optic axis in the superior-inferior plane was facilitated.

2.1.3 Electrophysiological Procedures

2.1.3.1 Preparation of animals. Preparation of the animals for recording was carried out under dim light (2 lux). Animals were anesthetized with sodium pentobarbitol (35-40 mg/kg, i.p.) and the pupils were dilated with Mydriacyl (tropicamide, 1%; Alcon Laboratories, Inc.) and Neo-Synephrine (phenylephrine HCl, 10%; Sterling Drug, Inc.). A topical anesthetic (proparacaine HCl, 0.5%) was applied to the cornea. Electrical responses were recorded through plastic contact lenses containing either platinum or Ag-AgCl electrodes. Electrical contact with the cornea was achieved by filling the lenses with an ophthalmic demulcent solution (hydroxypropylmethylcellulose, 2.5%) before placing them onto the corneas. A Ag-AgCl reference electrode was placed either on the scalp or was surgically implanted under the skin at the external canthus, and a ground electrode was attached to the animal's ear. Electrode gel (GE Jel; General Electric) was applied to the reference and ground electrodes before positioning them on the animal.

2.1.3.2 Light stimuli. The light stimuli used to evoke the electroretinograms (ERGs) were produced by a xenon lamp, Grass Photostimulator (PS2) with a stabilized power supply. Each flash stimulus was 25 μsec in duration. Adaptation light and flash stimuli were delivered to the rats' eyes by way of a Ganzfeld. A white hemisphere with an internal diameter of 30 cm served as the Ganzfeld. During adaptation and recording, the rat's head was positioned inside the Ganzfeld so that the eyes were approximately 2 cm from the inside surface of the hemisphere.

2.1.4 Statistical Methods

One way and two way analyses of variance and post-hoc Bonferroni t-tests and Duncan multiple range tests were executed using the facilities of the Northeast Regional Data Center on the University of Florida campus. The statistical procedures portion of the 1982 release of the SAS system was used in doing the statistical tests (Goodnight et al., 1982). For the analyses of variance, the GLM procedure, which analyzes data within the framework of general linear models, was used since there were an unequal number of observations per cell (unbalanced design). Post-hoc Bonferroni t-tests and Duncan multiple range tests were used to determine whether experimental group means were significantly different from the control group means.

Comparisons between two group means were made using t-tests for unmatched data which did not require the assumption of equal variance (Darlington, 1975).

Linear regression analyses were conducted using the linear regression program in the Ed-Sci statistics package for microcomputers (Ed-Sci Development, Modesto, CA).

2.2 Specific Methods

2.2.1 Determination of Light Damage Threshold

2.2.1.1 Experimental animals. All of the animals used in this experiment were Sprague-Dawley albino rats raised from birth under low intensity cyclic light (12:12

L:D; 6 ± 2 lux cage illumination], as described in Section 2.1.1.

2.2.1.2 Experimental lighting conditions. Four experimental light intensities were tested, viz. 1345, 270, 130 and 65 lux. When the 15-17 week old rats were transferred from the 6 lux rearing light condition to the experimental light condition, the transfer was effected at the start of a 12 hour light period. Animals exposed to the experimental light intensities were maintained on a 12:12 L:D cycle. For each of the intensities tested, groups of 6 rats were examined after various durations of light exposure in order to evaluate the effects of light on the physiological and morphological characteristics of the retina. The durations tested were 8 and 29 days exposure to 1345 lux, 1, 3, 7, 14, 28 and 59 days exposure to 270 lux, and 1, 3, 7, 14, 28, 59 and 85 days exposure to 130 or 65 lux. Control animals (n = 12) were maintained in rearing light conditions [12:12 L:D; 6 ± 2 lux cage illumination] and were examined throughout the 85 day testing period. Testing of the experimental and control animals occurred at the end of the 12 hour dark period which followed the final 12 hours of light exposure.

2.2.1.3 Electrophysiological procedures. All experimental and control animals were prepared for the recording sessions as described in Section 2.1.3.1. Electrical responses from both eyes were recorded differentially through plastic contact lenses containing platinum electrodes. A Ag-AgCl reference electrode was placed on the scalp between the eyes, and a ground electrode was attached to the animal's left ear.

Recording of the ERGs began after a 20 minute adaptation period to 0.1 lux. Animals were maintained at this adaptation level during subsequent recordings of the ERGs. The source of the light stimuli used to elicit the ERGs and the method used to present them to the animal are described in Section 3.1.3.2. Five flash intensities, measured as luminance of the Ganzfeld surface, were used in this experiment (-4.12, -3.48, -2.92, -2.28 and -1.48 log fL• sec) to elicit ERG responses. One exception to this procedure was in testing animals exposed to 1345 lux. Only three flash intensities were used (-3.48, -2.92 and -1.48 log fL•sec) to elicit responses from this group of animals. Four to six responses were recorded at each of the five flash intensities. The inter-stimulus interval was 30 seconds and two minutes were allowed to elapse before each new flash intensity was introduced. These conditions were sufficient to prevent light adaptation to the flash stimuli. Flash intensities were presented in ascending order, from lowest to highest intensity.

Electrical signals were amplified by a Tektronix Type 122 low level preamplifier (1000x; pass band 0.1 - 1 KHz) then averaged and stored by a Fabri-Tek 1052, 1024 point signal averager. Records of the responses were made using a

Clevite Brush Mark 250 chart recorder. Measurements of the b-wave amplitudes and latencies were made from the records of the responses using an image analyzing system which consisted of an Apple II+ computer, a Bausch & Lomb Houston Instruments Hipad digitizing tablet and Bioquant software (Digitizing Morphometry program; R&M Biometrics, Nashville, TN). Amplitudes of the b-waves were measured from the bottom of the a-wave to the peak of the b-wave. Latencies of the b-waves were measured as the time from stimulus onset to the peaks of the responses. The amplitude of the b-wave component of the computer averaged ERG responses was calculated for each animal at each of the five flash intensities tested.

2.2.1.4 Statistical analysis of electrophysiological data. Control animals were tested at various times throughout the experiment. Their amplitude and latency data showed no change with time, and so all of the control data collected at the various times were combined into a single control group.

One way analyses of variance (a total of 36) were performed to determine the effect of duration of experimental light exposure on the b-wave parameters (i.e. amplitude and latency). For each of the five flash intensities, and at each of the four experimental light intensities (1345, 270, 130 and 65 lux), the groups exposed for various lengths of time to experimental light were compared to the pooled control group. The significance of differences between group means were then assessed by post-hoc comparisons using Bonferroni t-tests with the significance level set at p=0.05 (see Section 2.1.4 for a description of the statistical tests used).

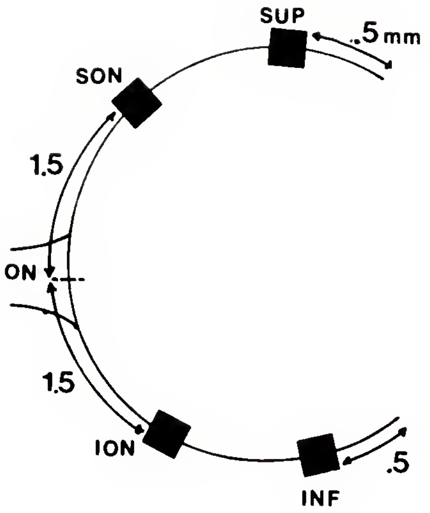
2.2.1.5 Morphometric analysis of retinal tissues. The eye tissues were prepared using the methods described in Section 2.1.2. Tissue sections, 1 μm thick, were cut along the superior-inferior vertical axis using a Sorvall Porter-Blum MT2-B ultra-microtome, mounted on slides and stained with a solution of 1% toluidine blue in 1% sodium borate in distilled water.

Histological sections taken from the central retina which included the optic nerve were analyzed using the image analyzing system described above (Section 2.2.1.3) in conjuction with a Nikon Labophot microscope, drawing tube and camera attachment. The light microscopic image of the tissue being examined was projected onto the digitizing tablet by the drawing tube. Retinal tissue measurements were made by tracing the projected areas on the tablet surface with an LED cursor (Bausch & Lomb) which subsequently fed the information into the computer.

Four regions along the superior-inferior length of each retina were analyzed for light-induced histological change. The four regions, each of which represented a 180 μm length of retina, were located (1) 500 μm inferior to the superior margin of the retina (SUP), (2) 1500 μm superior to the optic nerve head (SON), (3) 1500 μm inferior to the optic nerve head (ION) and (4) 500 μm superior to the inferior margin of the retina (INF). The region 1500 μm superior to the optic nerve head was included in the analysis since it has been shown to incur the greatest amount of damage following exposure to toxic light (Rapp and Williams, 1980). The widths of five retinal layers were measured in each region: (1) the pigment epithelium, measured from apical to basal borders; (2) the photoreceptor outer segments plus inner segments, which were measured from the external limiting membrane to the apical tips of the outer segments; (3) the outer nuclear layer, which was measured from the most vitread edge of the photoreceptor nuclei to the external limiting membrane; (4) the inner nuclear layer and (5) the inner plexiform layer, which was measured from the most vitread edge of the inner nuclear layer to the ganglion cell layer. The regions and retinal layers analyzed are diagrammed in Figure 2-1. Measurements were repeated three times in each region and were made at approximately 50 μm intervals along the 180 μm length of retina being examined. Mean widths of the individual layers were calculated by region for each retina examined and these values were used in further analyses of the data.

2.2.1.6 Statistical analysis of morphometric data. Retinas from control animals were examined at various times throughout the experiment. The widths of the individual retinal layers within each of the four regions examined showed no systematic change with time, and so all of the

Figure 2-1. Schematic drawings of the retinal regions and layers examined in the analyses of the effects of various intensities of light on the morphology of the albino rat retina. Four retinal regions were examined and their relative location along the superior-inferior length of the retina is shown on the left side of the figure. The widths of five retinal layers were measured in each of the four retinal regions. The diagram on the right side of the figure illustrates the boundaries of each of the layers measured. Abbreviations: PE - pigment epithelium; OS+IS - photoreceptor outer and inner segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer; SUP - superior; SON - superior optic nerve; ION - inferior optic nerve; INF - inferior.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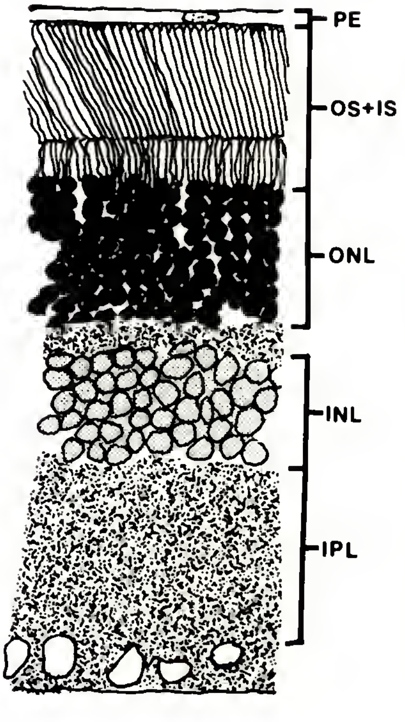
INF
.5


PE
OS+IS
ONL
INL
IPL

control data collected at the various times were combined into a single control group.

One way analyses of variance (a total of 80) were performed to determine the effect of duration of experimental light exposure on the widths of the various layers of the retina. For each of the four retinal regions, and at each of the four experimental light intensities (1345, 270, 130 and 65 lux), the groups exposed for various lengths of time to experimental light were compared to the pooled control group. The sigificance of differences between group means were then assessed by post-hoc comparisons using Bonferroni t-tests with the significance level set at p=0.05 (see Section 2.1.4 for a description of the statistical tests used).

2.2.1.7 Semi-quantitative analysis of retinal tissues. In addition to the morphometric analyses of the histological data, photographs of the retinas were evaluated for light-induced pathological changes using a numerical rating system adapted from that described by Currier et al. (1983). This type of analysis provided a means for distinguishing the more subtle pathologic changes observed to occur in the retinas of animals exposed to experimental light intensities approaching control level (6 lux), from artifact.

Four layers of the retina were analyzed using the scoring criteria listed in Table 2-1. The pigment epithelium and photoreceptor layers were the focus of the

TABLE 2-1

CRITERIA USED FOR SCORING HISTOLOGICAL CHANGES IN SPECIFIC LAYERS OF RETINA

CELL LAYER	CODE	DESCRIPTION OF TISSUE
PE	1	normal
	2	cells swollen, nuclei rounded, vacuolization
	3	absent
OS	1	normal
	2	vesiculated apical tips, swelling
	3	50% total outer segments gone, vesiculated outer segment membranes, macrophages present
	4	less than 25% total outer segments present, vesiculated outer segment membranes, macrophages present
	5	absent
IS	1	normal
	2	slight edema, abnormal spacing between inner segments
	3	edema, 50% total inner segments gone
	4	less than 25% inner segments present, edema
	5	absent
ONL (SUP and INF)	1	8-10 rows of nuclei, very few pyknotic
	2	8-10 rows of nuclei, spaces between cells, pyknosis
	3	6-7 rows of nuclei, spaces between cells, pyknosis
	4	3-5 rows of nuclei, spaces between cells, pyknosis
	5	0-2 rows of nuclei, pyknosis
ONL (SON and ION)	1	9-10 rows of nuclei. very few pyknotic
	2	9-10 rows of nuclei, spaces between cells, pyknosis
	3	7-8 rows of nuclei, spaces between cells, pyknosis
	4	3-6 rows of nuclei, spaces between cells, pyknosis
	5	0-2 rows of nuclei, pyknosis

analysis. The choice of which layers to examine was based on my observation and those of previous investigators that photoreceptor cells, which are comprised of outer segments, inner segments and nuclei, are very sensitive to toxic light and are often the first cells of the retina affected in the early stages of light damage (Grignolo et al., 1969; Hannson, 1970; Kuwabara, 1970; O'Steen and Lytle, 1971; O'Steen et al., 1972; Shear et al., 1973). Two sets of codes were used to evaluate the outer nuclear layer in order to adjust for the inherent regional differences in the number of nuclei found in this layer across the length of the retina. Photographs taken of the four retinal regions described previously (see Figure 2-1), including the pigment epithelium, the photoreceptor outer and inner segments and the outer nuclear layer, were examined and assigned scores by myself and by a second observer who was an experienced histologist but was otherwise unaware of the groups in this experiment. Four sets of scores, each set containing a score for each of the four retinal layers examined, were obtained from each observer for each retina analyzed.

2.2.1.8 Statistical analysis of semi-quantitative morphological data. Total scores for each of the four regions examined in each retina were calculated. The results obtained from each observer were averaged to yield a mean total histological score for each of the four regions of the retina analyzed following exposure to 1345, 270, 130 or 65 lux for variable lengths of time. In order to determine the effects of the duration of experimental light exposure on the morphology of the outer layers of the retina, t-tests were performed in which the mean regional histological scores obtained for control animals were compared to those obtained for the groups exposed for various lengths of time to experimental light (see Section 2.1.4 for a description of the statistical test used).

2.2.2 Effects of Dim Cyclic Light on the Pigment Epithelium

2.2.2.1 Experimental animals. Two groups of albino rats (Sprague-Dawley strain) and one group of pigmented rats (hooded Long-Evans strain) were used in this experiment. One group of albino rats (n=10) was raised from birth in the dark (dark-reared) and experienced only brief periods of minimal illumination (0.1 lux) during routine cleaning and feeding (three 5 minute periods per week). The other group of albino rats (n=11) was raised from birth under controlled low intensity cyclic lighting conditions (12:12 L:D; 6 ± 2 lux ambient cage illumination). Albino rats were 15-17 weeks old at the start of this experiment. Adult pigmented rats (n=10) were raised in a commercial facility, but upon arrival in our laboratory, were also housed under controlled lighting conditions (12:12 L:D; 6 ± 2 lux ambient cage illumination) (see Section 2.1.1 for a detailed description of animals and the housing and lighting conditions).

2.2.2.2 Electrophysiological procedures. All ERG recording sessions were performed during the first four hours of the 12 hour light period. Rats were prepared for the recording sessions as described in Section 2.1.3.1. Electrical responses were recorded differentially through a clear plastic contact lens containing a cylindrical (2 mm diameter) Ag-AgCl pellet electrode (In Vivo Metric Systems, Healdsburg, CA). A Ag-AgCl reference electrode was surgically placed under the skin at the external canthus and a ground electrode was attached to the animal's ear.

Two series of ERG recordings were made during the session. The first was conducted after the animal had been exposed to complete darkness for 20 minutes. The second was conducted after a 20 minute adaptation period to 0.1 lux. Animals were maintained at these adaptation levels during subsequent recordings of the ERG. The source of the light stimuli used to elicit the ERGs and the method used to present the light stimuli to the animal are described in Section 2.1.3.2. Three flash intensities, measured as luminance of the Ganzfeld surface, were used in this experiment (-1.48, -0.91 and -0.08 log fL•sec). Four responses were recorded at each of the three flash intensities following the two adaptation periods. The inter-stimulus interval was 30 seconds. Two minutes were allowed to elapse before each new flash intensity was introduced.

Electrical signals were pre-amplified by a Dynamics 890 d.c. amplifier (1000x; pass band d.c.-100 Hz) then averaged and stored by a Nicolet data processor (Model NIC-80). The amplitudes of the b-waves were measured on-line from the bottom of the a-wave to the peak of the b-wave. The amplitudes of the c-waves were measured on-line as positive deflections from the baseline determined prior to the flash. The amplitudes of the b- and c-wave components of a single response to each of the three flash intensities were calculated for each animal. The latencies of the b- and c-waves were measured as the time from stimulus onset to the peak of the responses. These measurements were facilitated by using a program available for the Nicolet data processor which allows the user to expand both axes of the signal stored in memory and locate maximum and minimum points in the signal using a cursor. Records of the responses were made using a Houston Instruments Omnigraphic 2000 x-y recorder.

2.2.2.3 Statistical analysis of electrophysiological data. Group means were determined for the amplitudes and latencies of the b- and c-wave components of the ERGs recorded in response to each of the three flash intensities tested. The mean amplitudes or latencies of the b- and c-wave components of the ERG were compared between experimental groups at each of the three flash intensities and two adaptation levels tested using six separate one way analyses of variance followed by Duncan's multiple range tests with the significance level set at p=0.05 (see Section 2.1.4 for a description of the statistical tests used).

2.2.2.4 Procedure for eliciting and measuring sodium azide-induced changes in the resting potential of the eye. Sodium azide was used to assess the physiological status of the pigment epithelium. The azide response was elicited by rapid injection of azide (40mM, 0.1 ml/kg) (Noell, 1952) through an indwelling catheter which had been implanted into the right external jugular vein 2-3 days beforehand. Catheters were constructed out of Silastic medical grade tubing (0.025" I.D. x 0.047" O.D. and 0.020" I.D. x 0.037" O.D.; Dow Corning), 21 gauge hypodermic needles (Becton-Dickinson) and Tygon microbore tubing (I.D. 0.020" x 0.060" O.D.; Norton Plastics and Synthetics Division). A 9 cm length of the smaller bore Silastic tubing was fitted with two 3 mm cuffs of the larger Silastic tubing. One cuff was placed at the end of the 9 cm length of tubing and the other was placed 3 cm in from the opposite end of the tubing. The catheter was completed by cutting and bending (angle = 90°) a 2 cm length of 21 gauge syringe needle (all metallic ends polished smooth), inserting one end into a 1 cm length of Tygon microbore tubing with pin stopper, and inserting the other end into the cuffed end of the 9 cm length of Silastic tubing. Prior to surgery, the catheter was rinsed in alchohol and filled with 0.9% saline.

The following surgical procedure was used to implant the catheter and represents a modification of the procedure described by Nicolaidis et al. (1974). Rats were anesthetized using Equithesin (2.5 ml/kg: 0.26M chloralhydrate, 0.18M magnesium sulfate, 19.5% (v/v) sodium pentobarbitol (1 grain/ml), 40% (v/v) propylene glycol and 10% (v/v) absolute ethyl alcohol in distilled water) and their eyes were shielded with opaque tape during the surgery which took approximately 30 minutes to perform. A small incision was made in the skin on the ventral side of the animal above the right external jugular vein, approximately 5 mm before it descends beneath the clavicle. The tissues surrounding the vein were gently teased away and two lengths of 3-0 surgical silk thread were positioned under the vein (5-10 mm apart). Prior to the insertion of the catheter into the vein, the rostral suture was tied off. A small incision was made in the vein between the silk threads and the free end of the catheter was pushed in up to the cuff (i.e. 3 cm). The tip of the catheter was thus positioned at the auricular cavity. The catheter was held in place by tying the second thread around the catheter and vein wall. An additional suture was made through the facia underneath the catheter and was tied around the catheter to secure it in place. The catheter was tested for patency by withdrawal of blood, and was then filled with a viscous heparinized solution of polyvinylpyrollidone (approx. 20% w/v in 0.9% saline). An incision was then made in the tissue covering the skull, the skull was scraped clean of connective tissue and three small jewelers screws (Lomat, Montreal) were inserted (<1 mm) into pretapped holes in the middle of the skull. The other end of the catheter was brought up to the back of the skull by threading it under the skin using a curved hemostat clamp and was cemented to the skull and screws using dental cement (Aneuroplastic powder #639897; L.D. Caulk, Co.) and Opotow Number "7" fast repair liquid hardener (Teledyne Getz).

Sodium azide salt was dissolved in water to a final concentration of 40mM and sufficient NaCl was added to make the final solution 0.15M in sodium and pH 7.4. Changes in the resting potential of the eye in response to an intravenous injection of sodium azide were measured in each animal at the end of the ERG recording session. The responses were recorded and plotted using the techniques described in Section 2.2.2.2. The amplitude of the azide response was taken as the maximum positive deflection of the resting potential from the baseline determined prior to administration of azide.

2.2.2.5 Statistical analysis of azide responses. Mean azide responses were determined for each of the three experimental groups. The mean amplitudes of the responses were compared using one way analysis of variance. The significance of differences between group means was then assessed by post-hoc comparisons using a Duncan's multiple range test (see Section 2.1.4 for a description of the statistical tests used).

2.2.2.6 Analysis of retinal tissue. Histology was performed on the eyes of six animals, three dark-reared albinos and three albinos raised under low intensity cyclic light. Eye tissue was prepared according to the procedures outlined in Section 2.1.2. Tissue sections, 1 μm thick, were cut along the superior-inferior vertical axis using a Sorvall Porter-Blum MT2-B ultra-microtome, mounted on slides and stained with a solution of 1% toluidine blue and 1% sodium borate in distilled water. Retinas taken from albino rats raised under low intensity cyclic light were examined using light microscopic techniques to determine whether morphological changes associated with light damage were present. The retinas taken from these animals were qualitatively compared to those obtained from dark-reared albino rats. Attention was focused on the retinal region extending from 700-2000 μm superior to the optic nerve head in the vertical meridian since it has been shown to be most sensitive to light damage (Rapp and Williams, 1980).

2.2.3 Effects of Toxic Light on Photoreceptor Renewal

2.2.3.1 Experimental animals. All of the animals used in this experiment were Sprague-Dawley albino rats raised from birth under low intensity cyclic light (12:12 L:D; 6 ± 2 lux cage illumination) (see Section 2.1.1 for detailed description of animals and housing and lighting conditions).

2.2.3.2 Experimental lighting conditions. Two experimental light intensities were tested, 130 and 270 lux. Animals exposed to the experimental light intensities were maintained on a 12:12 L:D cycle. For each of the intensities tested, two rats were examined after 1, 3 or 7 days light exposure in order to evaluate the effects of toxic light on the process of photoreceptor outer segment renewal. Control animals were maintained in rearing light conditions (12:12 L:D; 6 ± 2 lux ambient cage illumination). Two control animals were killed on each of the three days with the experimentals.

2.2.3.3 Method for administration of radiolabelled amino acids. A mixture of tritiated L-amino acids (ICN; specific activity 225-273 mCi/mg) was dried down under vacuum (Savant Speed Vac Concentrator) and redissolved in 0.5 ml 0.9% saline to a final activity level of 10 mCi/ml. The tritiated amino acid mixture was injected i.v. at a dosage of 10μCi/g body weight through an indwelling catheter which had been implanted into the right external jugular vein 2-3 days beforehand. Catheters were constructed and surgically implanted according to the procedures outlined in Section 2.2.2.3.

All animals were injected at the end of the first 12 hours of exposure to the experimental light intensity being tested. Animals were exposed to the experimental light conditions for 1, 3 or 7 days and were sacrificed at the end of the 12 hour dark period which followed the final 12 hours of light exposure.

2.2.3.4 Procedures for preparing light microscopic autoradiograms. Animals were injected with an overdose of sodium pentobarbitol. A suture was placed at the dorsal most aspect of each eye (for orientation) and the eyes were enucleated. A small slit was made at the ora serrata and the eyes were immersed in fixative and refrigerated overnight (see Section 2.1.2.1). The eye tissues were dehydrated and embedded in JB-4 plastic using the methods described in Section 2.1.2.2. Tissue sections, 1 µm thick, were cut along the superior-inferior vertical axis using a Sorvall Porter-Blum MT2-B ultra-microtome and mounted on glass slides.

Autoradiograms were prepared by dipping the slides in Kodak NTB-2 bulk emulsion (Eastman Kodak Co., Rochester, NY) which was diluted 1:1 with distilled water at a temperature of 40°C. The slides were placed in a rack and allowed to dry for 1 hour before placing them into opaque slide boxes containing dessicant (silica gel). The preparations were exposed for 6 weeks at 4-7°C, developed in Kodak Dektol (1:1 dilution with distilled water) for 2 minutes and fixed in 30% sodium thiosulfate for 5 minutes after rinsing them in distilled water. All solutions were maintained at 18°C. After development and fixation, the slides were washed in three changes of distilled water for 15 minutes and the tissue sections were stained with 0.1% toluidine blue in 1% sodium borate heated to 60°C.

2.2.3.5 Analysis of autoradiograms. Autoradiograms were analyzed using a video image analyzing system which consisted of an Apple IIe computer, a Bausch & Lomb Houston Instruments Hipad digitizing tablet, Bioquant software (R&M Biometrics, Nashville, TN) and a Nikon Labophot microscope.

The microscope was fitted with a Mti 65 video camera. Dark-field microscopic images transmitted by the video camera were analyzed to determine (1) the position of the developed silver grains in the nuclear tracking emulsion overlying the retinal tissue relative to the outer segments of the photoreceptors and (2) silver grain density over the photoreceptor outer segment layer.

Estimates of the rates of membrane addition to the outer segment for the control and experimental groups were determined from linear regression equations calculated for the displacement of the radioactive band within the photoreceptor outer segment layer as a function of time. The position of the radioactive band within the photoreceptor outer segment layer was measured in micrometers from the base of the photoreceptor outer segments to the scleral edge of the silver grain band and time was expressed as elapsed time (hours) from the injection of the radioisotope to sacrifice of the animal. Radioactive band position measurements were repeated three times in the SON region of each retina.

Grain density, measured in terms of pixels per 1000 μm^2 was determined over the photoreceptor outer segment layer for each of the experimental and control retinas analyzed. Three estimates of grain density were made in the SON region of each retina analyzed.

2.2.3.6 Statistical analysis. Linear regression analyses of the experimental and control data for the displacement of the radioactive band within the photoreceptor outer segment layer as a function of time were carried out and rates of membrane renewal were determined from the regression equations obtained from the analyses. Two way analyses of variance were used to determine the effects of treatment and time on radioactive band displacement within the outer segments of the photoreceptors and on the density of silver grains overlying the photoreceptor outer segment layer of the retina. Section 2.1.4 contains a description of the statistical tests used.

CHAPTER 3
DETERMINATION OF LIGHT DAMAGE THRESHOLD

3.1 Introduction

Most light damage studies to date have investigated the effects of short-term constant light exposure on retinal morphology and function. Very few studies have investigated the effects of cyclic lighting regimes on the retina. The small amount of information which is available from these studies clearly indicates that cyclic light environments are capable of inducing retinal degeneration. A major point of disagreement between these studies concerns the intensity of light which produces retinal damage under these conditions.

The most thorough study to date concerning the effects of long-term cyclic lighting on the retina has been conducted by Weisse et al. (1974). These investigators exposed 6-week-old albino rats (SPF strain) to light intensities ranging from 32-237 lux (12:12 L:D) and examined the histology of the retinas of these animals 2, 2.5 and 2.9 years after light exposure was begun. After accounting for the age-related morphological changes which occur in the retina, they found that intensities as low as 32 lux produce atrophy of the photoreceptor layer, partial degeneration of the nuclei within the inner nuclear layer, and changes in the vascular pattern within the retina.

Greenman et al. (1982) have recently conducted a study similar to the Weisse et al. (1974) investigation. In their study, mice were exposed (BALB/cStCrlfC3Hf/Nctr strain) to light intensities ranging from approximately 86-2150 lux (12:12 L:D) for up to 33 months. The results of their study showed that retinal degeneration was restricted primarily to the photoreceptor layer and that all experimental light intensities produced some cases of retinal atrophy. Damage comparable to that observed in the Weisse et al. (1974) study was, however, found most often following exposures to 2150 lux. There is approximately a ten-fold discrepancy in the range of light intensities which the results of these studies suggest produce similar levels of damage to the retina.

The results of several studies concerning the short-term effects of cyclic light exposure on the retina further point out inconsistencies present in this literature. Results of a study conducted by Stötzer et al. (1970) suggest that light intensities of 60 lux (12:12 L:D) produce degenerative changes in the albino rat retina (SPF strain) in only 13 weeks. O'Steen et al. (1972) have reported that 194 lux (14:10 L:D) presented for 4-6 months does not cause retinal degeneration in albino rats. Carter-Dawson et al. (1982) have reported that 107 lux (12:12 L:D) presented for approximately 6 months does not cause retinal degeneration in albino rats (Sprague-Dawley strain). The results of a study conducted by Bons et al. (1977) suggest that the retinas of albino rats are not damaged after 4 weeks exposure to 3000 lux (12:12 L:D); however, degeneration was evident in the retinas of these animals at lower light intensities (1200 lux) if the duration of the light portion of the light cycle was extended to 14 hours. Finally, Williams et al. (1985) have reported that fewer than 3 days exposure to 133 lux (12:12 L:D) causes a significant reduction in the number of photoreceptor nuclei in the retinas of albino rats (Sprague-Dawley strain) raised under 3 lux (12:12 L:D).

Collectively, the results of these studies clearly show that very low light intensities are capable of inducing retinal damage in a cyclic light exposure paradigm. The apparent discrepancies between the results of these studies may be due to several factors. Factors which have been shown to influence the extent of the structural changes associated with retinal light damage are (1) the intensity and wavelength of the light (Gorn and Kuwabara, 1967; Noell et al., 1966), (2) the duration of light exposure (Fifkova, 1972; Rapp and Williams, 1980; Weisse et al., 1974), (3) the species of animal (Kuwabara and Gorn, 1968; Lanum, 1978), (4) the age and body temperature of the animal (Noell et al., 1966; O'Steen et al., 1974), (5) animal pigmentation (Rapp and Williams, 1980), and (6) the light history of the animal prior to light exposure (Noell, 1979). Currently, our understanding of these factors and their possible interactions as they relate to retinal light damage is relatively poor. Even if the factors most responsible for the discrepancies in the existing light damage literature were known, integration of the results of these studies would be extremely difficult since many of the published reports in this area do not include information relevant to these issues.

The aim of the present study was to determine the minimum light intensity which produces retinal damage in albino rats maintained under carefully controlled cyclic lighting conditions prior to and throughout 3 months exposure to various experimental light intensities. All animals were raised to 15 weeks of age under 6 lux (12:12 L:D) and were then transferred to 65, 130, 270 or 1345 lux (12:12 L:D). Control animals remained under 6 lux (12:12 L:D). After various lengths of exposure to the experimental light intensities, the conditions of the animals' retinas were assessed using electrophysiological and anatomical techniques. The amplitudes and latencies of the b-wave components of the ERGs recorded from the albino rats exposed to the experimental light intensities were compared to those recorded from the control animals maintained under the rearing light conditions. Similarly, the morphological changes observed in the retinas of the experimental animals were compared to those found in the retinas of the control animals. Retinas were considered to be damaged if either the physiological or the morphological evaluations of the retinas of the experimental animals were found to be significantly different from the control group evaluations following three or more consecutive exposure periods.

3.2 Results

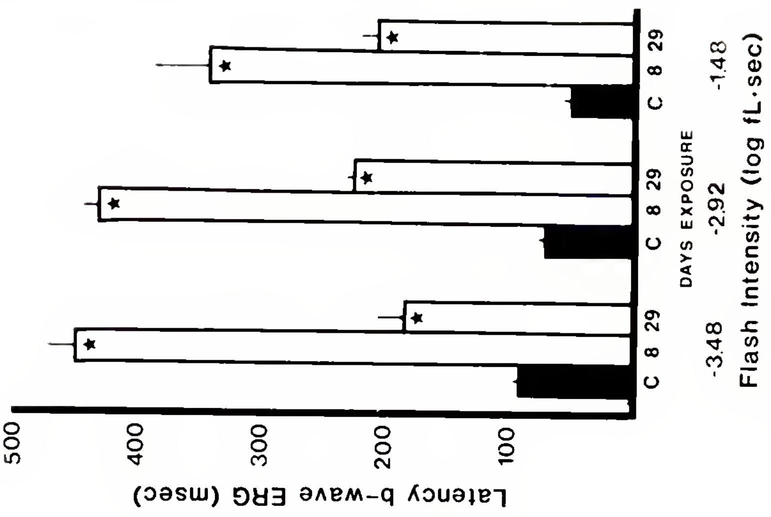
3.2.1 Electrophysiological Results

3.2.1.1 Effects of 1345 lux on ERG b-waves. The b-wave components of ERGs recorded from albino rats raised from birth under low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination) and subsequently exposed to 1345 lux (12:12 L:D) were significantly smaller and had significantly longer latencies than those recorded from control animals. Figure 3-1 summarizes the b-wave amplitude and latency data obtained from animals exposed to 1345 lux for either 8 or 29 days. Mean amplitude and latency data were compared between control and experimental groups at each of the three flash intensities using six separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at p=0.05.

The results of the between group comparisons of the amplitudes of the b-waves elicited to the three flash intensities showed that there was a significant between groups effect in all cases (see Table 3-1 for ANOVA results). Post-hoc tests on the means revealed that the b-waves recorded from animals exposed to 1345 lux for 8 or 29 days were significantly smaller than those recorded from the control animals but were not significantly different from each other.

The between group comparisons of the latencies of the b-waves elicited to the three flash intensities were also found to be significant in all cases (see Table 3-2 for

Figure 3-1. Amplitudes and latencies of the b-wave component of the ERGs recorded from the retinas of animals exposed to 1345 lux for 8 or 29 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). Responses were recorded at five flash intensity levels. Each bar represents the mean (+SE) group response. Control group (C) responses are represented by shaded bars. Experimental group means marked with an asterisk (*) were significantly different ($p=0.05$) from the corresponding control group means.


Latency b-wave ERG (msec)
500
400
300
200
100
C 8 29
C 8 29
C 8 29
DAYS EXPOSURE
-3.48
-2.92
-1.48
Flash Intensity (log fL·sec)

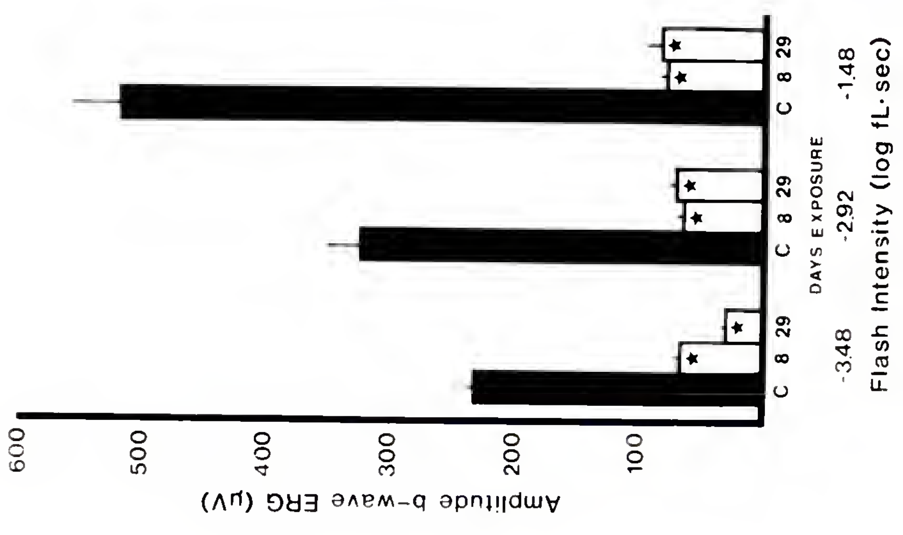

Amplitude b-wave ERG (µV)
600
500
400
300
200
100
C 8 29
C 8 29
C 8 29
DAYS EXPOSURE
-3.48
-2.92
-1.48
Flash Intensity (log fL·sec)

TABLE 3-1

COMPARISONS OF MEAN ERG B-WAVE AMPLITUDES RECORDED FROM CONTROL AND EXPERIMENTAL ANIMALS EXPOSED TO 1345, 270, 130 OR 65 LUX ACROSS THE VARIOUS DAYS OF EXPOSURE TESTED AND WITHIN THE FIVE FLASH INTENSITIES USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

LIGHT INTENSITY	(df)	FLASH INTENSITY (log fL·sec) -4.12	-3.48	-2.92	-2.28	-1.48
1345 lux	(2,23)	----	27.22†***	26.95 ***	----	47.00 ***
270 lux	(6,74)	11.53 ***	11.24 ***	8.95 ***	11.27 ***	13.89 ***
130 lux	(7,90)	3.21 *	1.33	1.12	1.98	.77
65 lux	(7,92)	4.21 **	6.54 ***	4.34 **	7.43 ***	5.78 ***

†ANOVA F value
* p<.01
** p<.001
*** p<.0001

ANOVA results]. Post-hoc tests on the mean latency values revealed that latencies of ERG b-waves recorded from animals exposed to 1345 lux for 8 or 29 days were significantly greater than those of the control animals. The latencies of the b-waves recorded from animals exposed to 1345 lux for 8 days were significantly greater than those recorded from animals exposed to 1345 lux for 29 days.

3.2.1.2 Effects of 270 lux on ERG b-waves. The b-wave components of ERGs recorded from albino rats raised from birth under low intensity light [12:12 L:D; 6 lux ambient cage illumination] and subsequently exposed to three or more days of 270 lux [12:12 L:D] were, in general, significantly smaller and had significantly longer latencies than those recorded from control animals. Figure 3-2 summarizes the b-wave amplitude and latency data obtained from animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days. Mean amplitude and latency data were compared between control and experimental groups at each of the five flash intensities using 10 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at p=0.05.

The results of the between group comparisons of the amplitudes of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect in all cases [see Table 3-1 for ANOVA results]. Post-hoc tests on the means revealed that the amplitudes of the b-waves recorded from animals exposed to

TABLE 3-2

COMPARISONS OF THE MEAN LATENCIES OF THE B-WAVES RECORDED FROM CONTROL AND EXPERIMENTAL ANIMALS EXPOSED TO 1345, 270, 130 OR 65 LUX ACROSS THE VARIOUS DAYS OF EXPOSURE TESTED AND WITHIN THE FIVE FLASH INTENSITIES USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

LIGHT INTENSITY	(df)	FLASH INTENSITY (log fL·sec)				
		-4.12	-3.48	-2.92	-2.28	-1.48
1345 lux	(2,23)	----	229.36† ***	1219.7 ***	----	119.56 ***
270 lux	(7,73)	17.41 ***	35.87 ***	42.08 ***	28.69 ***	21.37 ***
130 lux	(7,90)	4.34 **	6.42 ***	6.14 ***	8.39 ***	8.71 ***
65 lux	(7,91)	3.46 *	1.33	6.60 ***	2.94 *	1.22

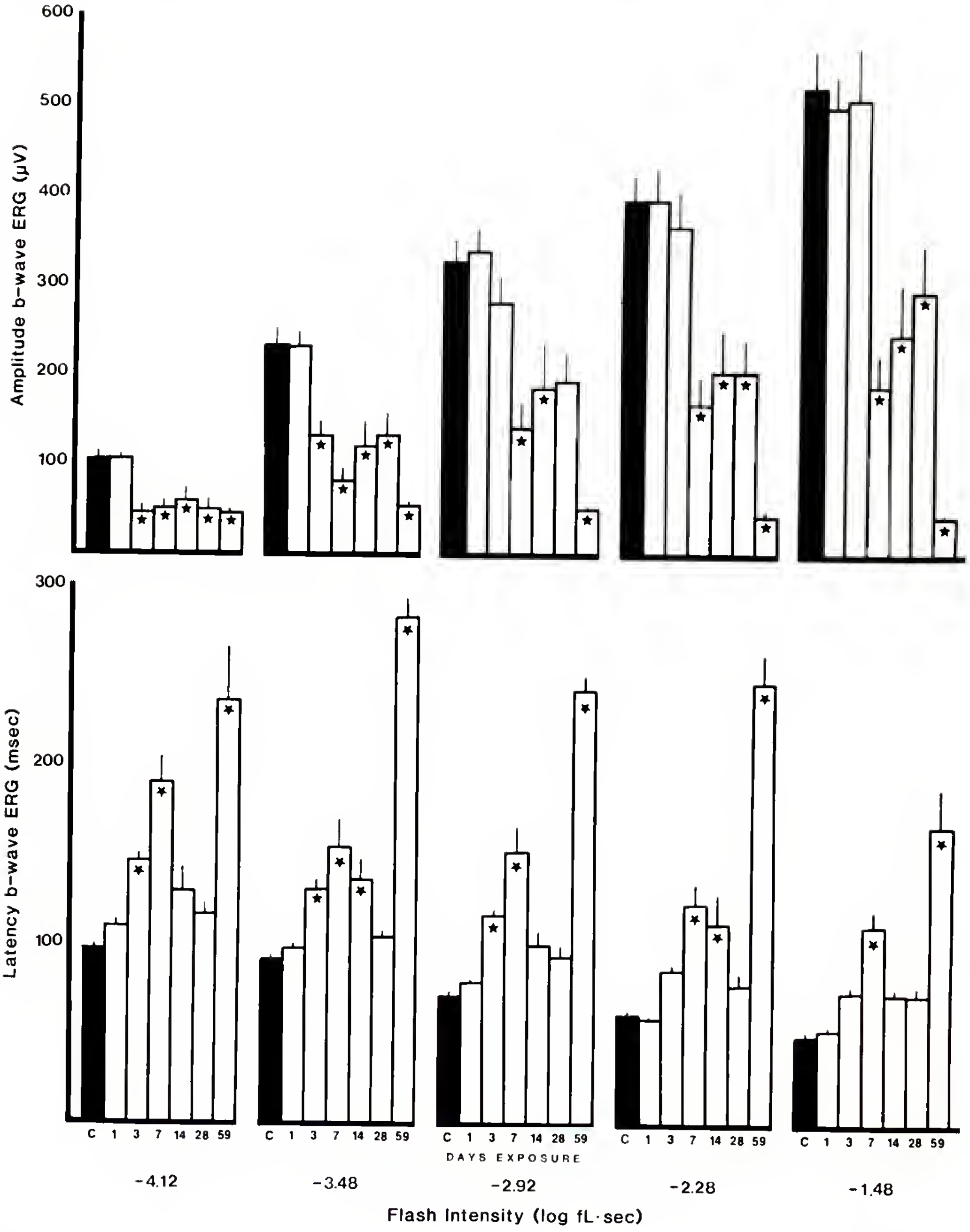
† ANOVA F value

* p<.01

** p<.001

*** p<.0001

Figure 3-2. Amplitudes and latencies of the b-wave component of the ERGs recorded from the retinas of animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). Responses were recorded at five flash intensity levels. Each bar represents the mean (+SE) group response. Control group (C) responses are represented by shaded bars. Experimental group means marked with an asterisk (*) were significantly different (p=0.05) from the corresponding control group means.


600
500
400
300
200
100
Amplitude b-wave ERG (μV)
300
200
100
Latency b-wave ERG (msec)
C 1 3 7 14 28 59
DAYS EXPOSURE
-4.12
-3.48
-2.92
-2.28
-1.48
Flash Intensity (log fL·sec)

270 lux for 3, 7, 14, 28 or 59 days were significantly smaller than control values in response to the two lowest flash intensities tested (-4.12 and -3.48 log fL•sec). Corresponding responses recorded from animals exposed to 270 lux for 1 day were not different from control responses. The results of post-hoc tests on the mean amplitudes of the b-waves elicited by the higher flash intensities (-2.92, -2.28 and -1.48 log fL•sec) revealed that the responses recorded from animals exposed to 270 lux for 7, 14, 28 or 59 days were significantly smaller than control responses. Responses recorded from animals exposed to 270 lux for 1 or 3 days were not different from control responses.

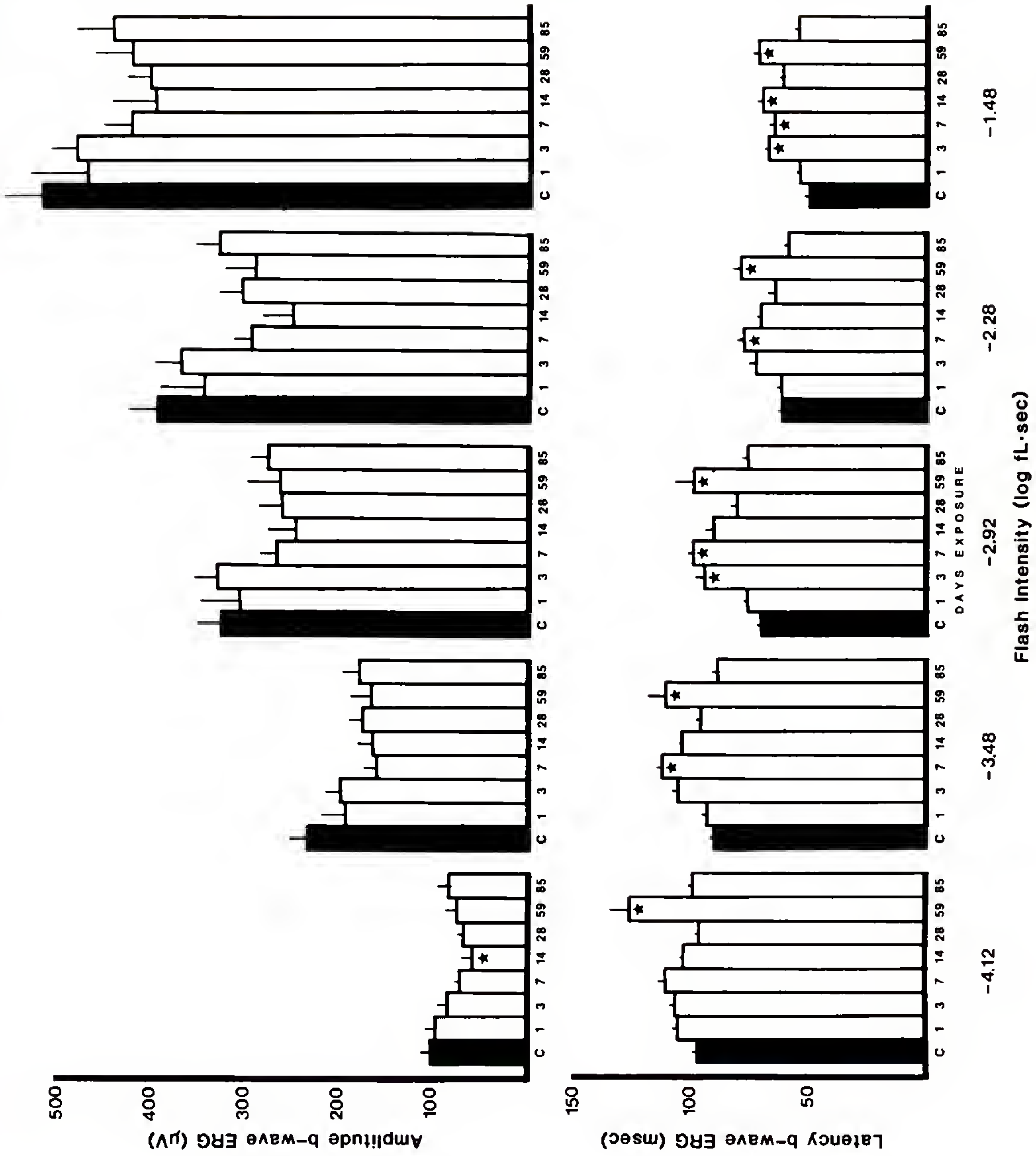
The results of the between group comparisons of the latencies of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect in all cases (see Table 3-2 for ANOVA results). Post-hoc tests on mean b-wave latency values for animals exposed to 270 lux for 3, 7 or 59 days revealed that the mean latency values calculated for these animals were significantly greater than those calculated for control animals in response to flash intensities of -4.12 and -2.92 log fL•sec. Mean b-wave latency values for animals exposed to 270 lux for 1, 14 or 28 days were not different from control values at these two flash intensities. Post-hoc tests on mean latency values for experimental and control animals in response to the -3.48 log fL•sec flash revealed that mean values calculated for animals exposed to 270 lux for 3, 7, 14 or 59 days were significantly greater than the mean control value. Mean latency values obtained for animals exposed to 270 lux for 1 or 28 days were not different from the mean control value at this flash intensity. Post-hoc tests on mean b-wave latency values for experimental and control animals in response to the -2.28 log fL•sec flash revealed that mean values calculated for animals exposed to 270 lux for 7, 14 or 59 days were significantly greater than the mean control value. In this case, the mean latency values obtained for animals exposed to 270 lux for 1, 3 or 28 days were not different from the mean control value. Finally, post-hoc tests on mean latency values for experimental and control animals in response to the -1.48 log fL•sec flash revealed that mean latency values obtained for animals exposed to 270 lux for 7 or 59 days were significantly greater than the mean control value. Mean latency values calculated for animals exposed to 270 lux for 1, 3, 14 or 28 days were not different from the mean control value.

3.2.1.3 Effects of 130 lux on ERG b-waves. The amplitudes of the b-wave components of ERGs recorded from albino rats raised from birth under low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination) and subsequently exposed to 130 lux (12:12 L:D) were not, in general, different from those recorded from controls. The latencies of the b-waves recorded from albino rats exposed to 130 lux were, in some cases, greater than the latencies of the b-waves recorded from the control animals. Figure 3-3 summarizes the b-wave amplitude and latency data obtained from animals exposed to 130 lux for 1, 3, 7, 14, 28, 56 or 85 days. Mean amplitude and latency data were compared between control and experimental groups at each of the five flash intensities using 10 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at p=0.05.

The results of the between group comparisons of the amplitudes of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect at only one flash intensity, the -4.12 log fL• sec flash (see Table 3-1 for ANOVA results). Post-hoc tests on the mean amplitudes of the b-waves recorded from experimental and control animals in response to the -4.12 log fL•sec flash revealed that the responses recorded from animals exposed to 130 lux for 14 days were significantly smaller than the control responses. Amplitudes of responses recorded from animals exposed to 130 lux for 1, 3, 7, 28, 56 or 85 days were not different from the amplitudes of control responses at this flash intensity.

The results of the between group comparisons of the latencies of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect in all cases (see Table 3-2 for ANOVA results). Post-hoc tests on the mean b-wave latency values calculated for animals exposed to 130 lux for 7 or 56 days

Figure 3-3. Amplitudes and latencies of the b-wave component of the ERGs recorded from the retinas of animals exposed to 130 lux for 1, 3, 7, 14, 28, 59 or 85 days [12:12 L:D]. The control group was maintained under 6 lux [12:12 L:D]. Responses were recorded at five flash intensity levels. Each bar represents the mean [+SE] group response. Control group [C] responses are represented by shaded bars. Experimental group means marked with an asterisk [*] were significantly different [$p=0.05$] from the corresponding control group means.

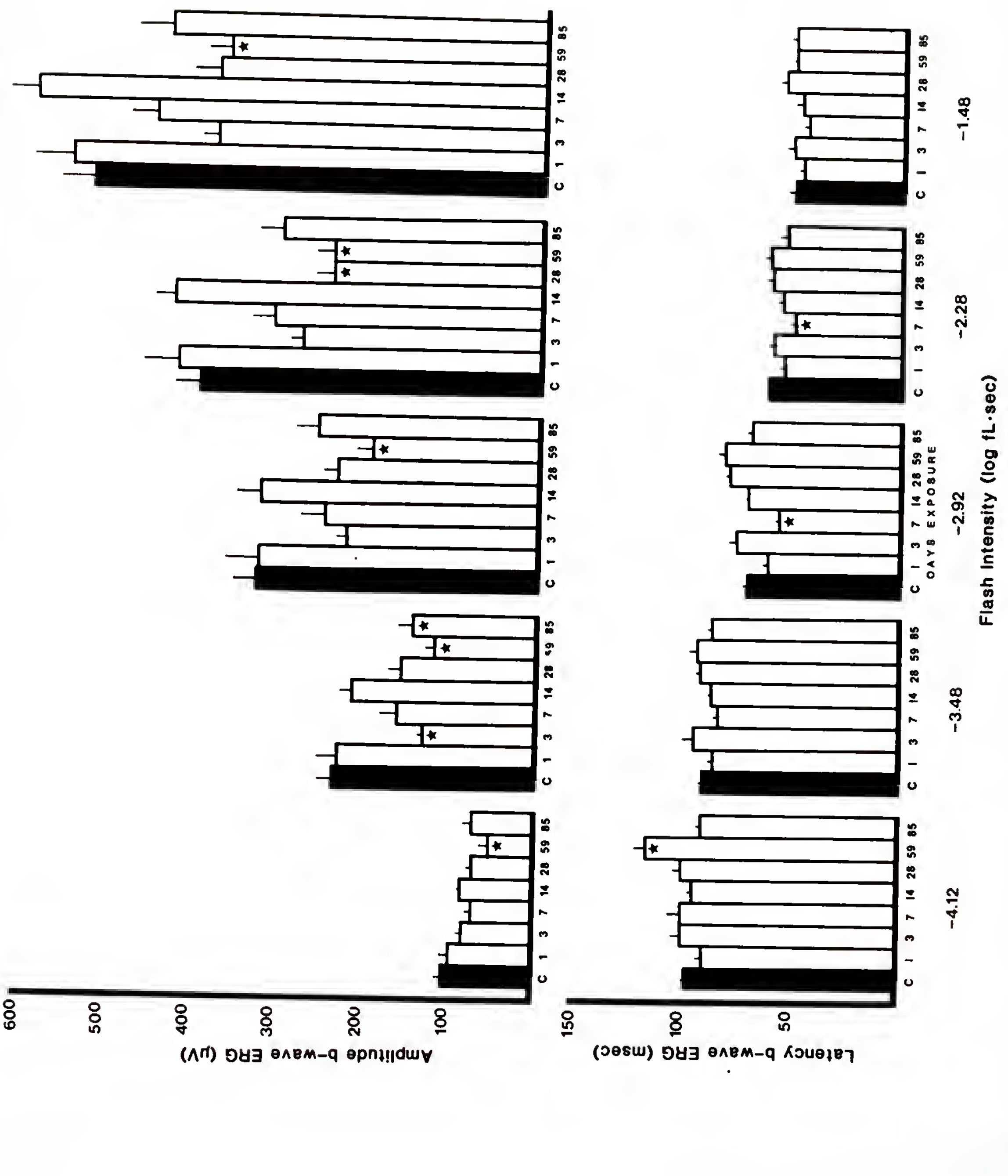

Amplitude b-wave ERG (µV)
500
400
300
200
100
Latency b-wave ERG (msec)
150
100
50
C 1 3 7 14 28 59 85
-4.12
-3.48
-2.92
-2.28
-1.48
DAYS EXPOSURE
Flash Intensity (log fL·sec)

were signifiantly greater than mean latency values obtained for control animals in response to flash intensities of -3.48 and -2.28 log fL•sec. Mean latency values for animals exposed to 130 lux for 1, 3, 14, 28 or 85 days were not different from mean control values at these two flash intensities. Post-hoc tests on mean latency values calculated for experimental and control animals in response to the -4.12 log fL•sec flash revealed that the mean value obtained for animals exposed to 130 lux for 56 days was significantly greater than the corresponding mean control value. Mean latency values for animals exposed to 130 lux for 1, 3, 7, 14, 28 or 85 days were not different from the mean control value. Post-hoc tests on the mean latency values calculated for experimental and control animals in response to the -2.92 log fL•sec flash revealed that the mean values obtained for animals exposed to 130 lux for 3, 7, or 56 days were significantly greater than the corresponding mean control value. Mean latency values for animals exposed to 130 lux for 1, 14, 28 or 85 days were not different from the mean control value. Finally, post-hoc tests on mean latency values obtained for the experimental and control animals in response to the -1.48 log fL•sec flash revealed that values obtained for animals exposed to 130 lux for 3, 7, 14 or 56 days were significantly greater than the corresponding mean control value. Mean latency values for animals exposed to 130 lux for 1, 28 or 85 days were not different from the mean control value.

3.2.1.4 Effects of 65 lux on ERG b-waves. The amplitudes of the b-wave components of the ERGs recorded from albino rats raised from birth under low intensity cyclic light [12:12 L:D; 6 lux ambient cage illumination] and subsequently exposed to 65 lux [12:12 L:D] were, in some cases, significantly smaller than the amplitudes of the corresponding control responses. The latencies of the b-wave components of the ERGs recorded from the animals exposed to 65 lux were, in general, not different from the latencies of the corresponding control responses. Figure 3-4 summarizes the b-wave amplitude and latency data obtained from animals exposed to 65 lux for 1, 3, 7, 14, 28, 56 or 85 days. Mean amplitude and latency data were compared between control and experimental groups at each of the five flash intensities using ten separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at $p=0.05$.

The results of the between group comparisons of the amplitudes of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect in all cases [see Table 3-1 for ANOVA results]. Post-hoc tests on the mean amplitudes of b-waves recorded from animals exposed to 65 lux for 56 days in response to flash intensities of -4.12, -2.92 and -1.48 log fL•sec revealed that the responses recorded from these animals were significantly smaller than those recorded from control animals. Responses recorded from animals exposed to

Figure 3-4. Amplitudes and latencies of the b-wave component of the ERGs recorded from the retinas of animals exposed to 65 lux for 1, 3, 7, 14, 28, 59 or 85 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). Responses were recorded at five flash intensity levels. Each bar represents the mean (+SE) group response. Control group (C) responses are represented by shaded bars. Experimental group means marked with an asterisk (*) were significantly different ($p=0.05$) from the corresponding control group means.


Amplitude b-wave ERG (µV)
600
500
400
300
200
100
Latency b-wave ERG (msec)
150
100
50
C 1 3 7 14 28 59 85
DAYS EXPOSURE
-4.12
-3.48
-2.92
-2.28
-1.48
Flash Intensity (log fL·sec)

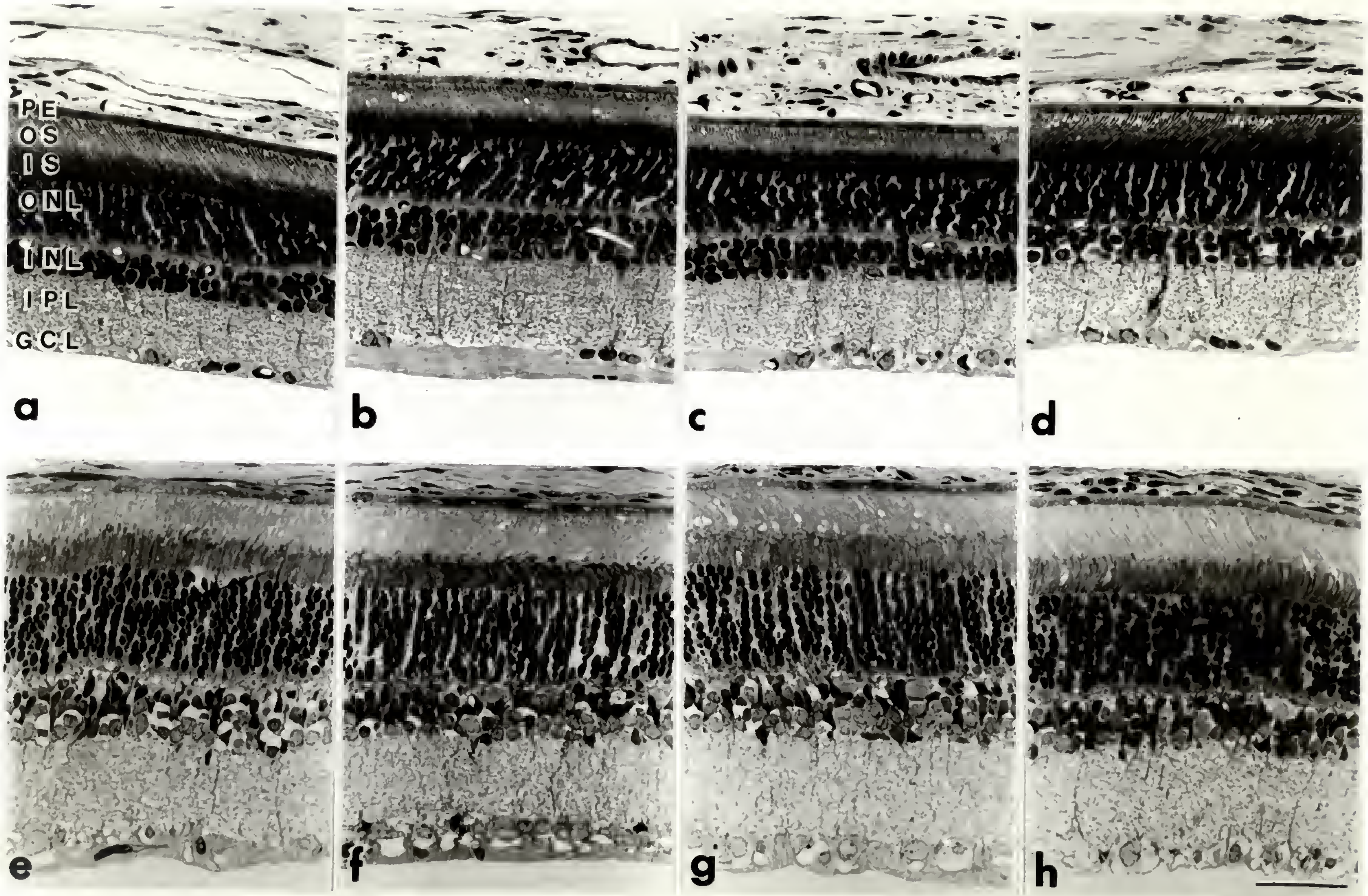
65 lux for 1, 3, 7, 14, 28 or 85 days were not different from control responses at these three flash intensities. Post-hoc tests on the mean amplitudes of the b-waves recorded from experimental and control animals in response to the -3.48 log fL•sec flash revealed that the responses recorded from animals exposed to 65 lux for 3, 56 or 85 days were significantly smaller than the control responses. Responses recorded from animals exposed to 65 lux for 1, 7, 14 or 28 days were not different from control responses. Finally, post-hoc tests on the mean amplitudes of the b-waves recorded from experimental and control animals in response to the -2.28 log fL•sec flash revealed that responses recorded from animals exposed to 65 lux for 28 or 56 days were significantly smaller than control responses. Responses recorded from animals exposed to 65 lux for 1, 3, 7, 14 or 85 days were not different from control responses.

The results of the between group comparisons of the latencies of the b-waves elicited to the five flash intensities showed that there was a significant between groups effect at three flash intensities, the -4.12, -2.92 and -2.28 log fL•sec flashes. Post-hoc tests revealed that the mean latency values calculated for animals exposed to 65 lux for 7 days in response to flash intensities of -2.92 and -2.28 log fL•sec were significantly less than those obtained for control animals. Mean latency values for animals exposed to 65 lux for 1, 3, 14, 28, 56 or 85 days were not different from mean control values at these two flash intensities. Post-hoc tests on mean latency values calculated for experimental and control animals in response to the -4.12 log fL•sec flash revealed that the mean value obtained for the animals exposed to 65 lux for 56 days was significantly greater than the mean control value. In this case, mean latency values obtained for animals exposed to 65 lux for 1, 3, 7, 14, 28 or 85 days were not different from the mean control value.

3.2.2 Histological Results

3.2.2.1 Retinal morphology of control animals and histological variability. Retinas from control animals were examined at various times throughout the experiment. While no significant changes in the widths of the individual retinal layers within each of the four regions were found with time, there was some variability observed between animals in the overall appearance of their retinas. This variability was primarily due to differences in the overall thickness of the retinas. An example of the variablity observed between control retinas is shown in Figure 3-5. The retina shown in the top portion of the figure (Figure 3-5, a-d) provides an example of the histological profile observed in 50% of the control retinas examined (5 out of 10 cases). Control retinas of this type exhibited compact nuclear and fiber layers within each of the four regions examined. The outer nuclear layer typically contained 8-10 rows of nuclei and the remaining layers of the retina appeared normal. The retina shown in the bottom portion of

Figure 3-5. Light photomicrographs of retinal tissue taken from two control animals maintained under 6 lux (12:12 L:D). The histology shown for each animal is representative of 50% of the cases examined (5 out of 10). Panels a-d are from one animal and panels e-h are from the other. Panels a and e were taken of the SUP retinal region, b and f were taken of the SON retinal region, c and g were taken of the ION retinal region and d and h were taken of the INF retinal region. A description of these retinal regions may be found in Section 2.2.1.5. Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; IS - photoreceptor inner segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer; GCL - ganglion cell layer. Calibration bar = 150 µm.


PE
OS
IS
ONL
INL
IPL
GCL
a
b
c
d
e
f
g
h

the figure (Figure 3-5, e-h) provides an example of the other 50% of the cases. In these retinas, there appears to be a greater amount of extracellular space between the nuclei which comprise the outer and inner nuclear layers and within the fiber layers. The number of rows of nuclei in the outer nuclear layer remains approximately 8-10. The reason for this variability in the overall thickness of the control retinas is not known, although several factors could be responsible.

Tissue fixation is the most obvious factor. Inadequate fixation due to collapsed blood vessels or slow fixative penetration may have contributed to the variability seen in the retinal tissue. If there was an occlusion of the retinal vasculature during perfusion, one would expect to find red blood cells in the vessels of the fixed tissue. Red blood cells were present in the vessels of some of the retinal tissue which appeared swollen and were also occasionally observed in tissue which did not appear swollen, albeit in smaller numbers. Tissue autolysis, as evidenced by poor tissue staining and loss of histological detail, was observed in only a few cases and was not severe. This histological evidence provides some support for the idea that inadequate fixation may have contributed to the observed histological variability in control retinas.

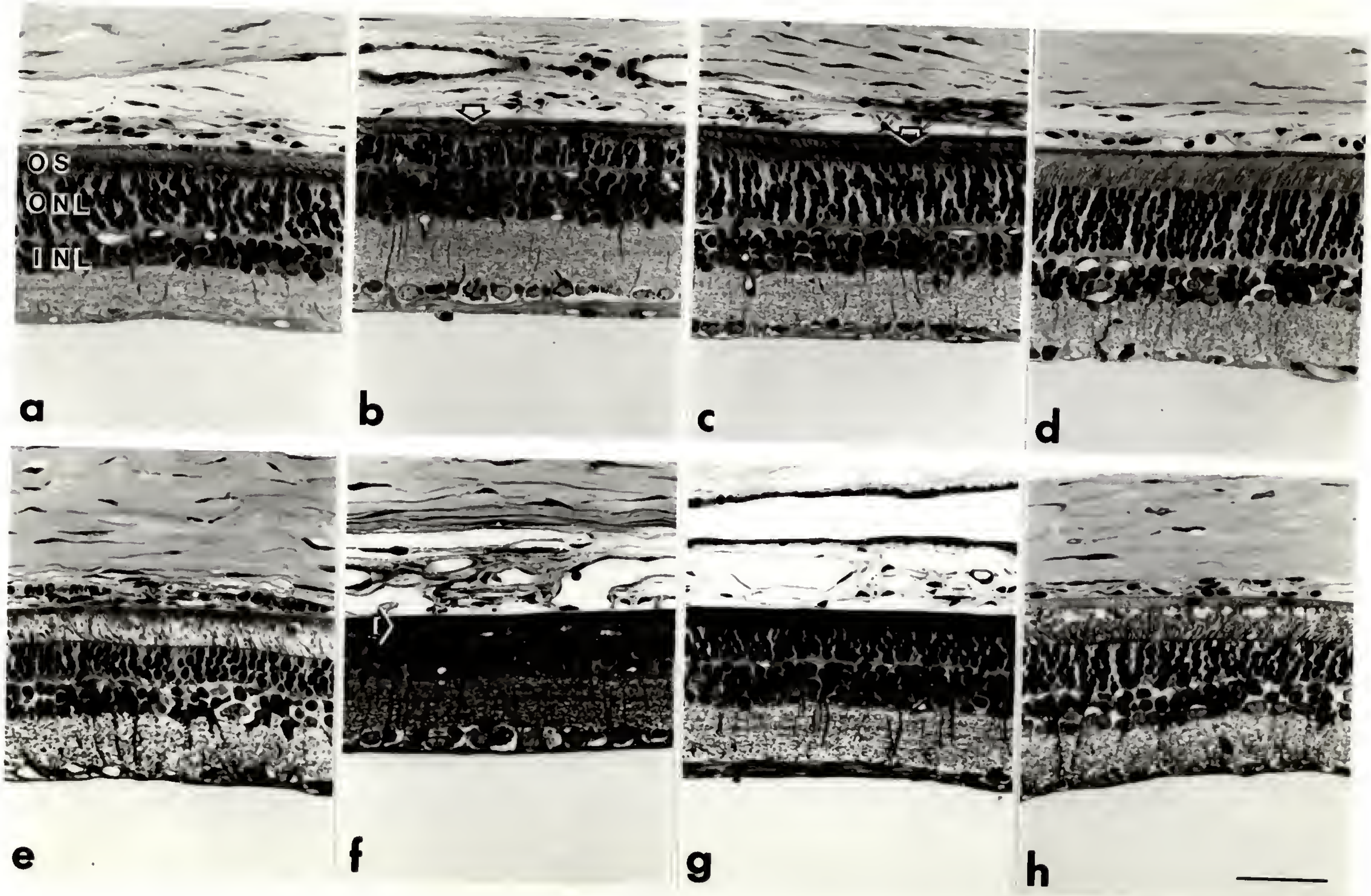
Mechanical deformation of the tissue during the embedding and sectioning processes may have contributed to the variability in the appearance of the retinal tissue. If mechanical deformation was a factor, evidence for it should have been apparent in the layer of the retina containing the fragile membranous photoreceptor outer segments. Evidence for shearing or compression was found in some of the retinas. For example, the junctions between the inner and outer segments of the photoreceptor cells form approximately a 100° angle in panels f and h of Figure 3-5 which implies that shearing of the tissue has taken place during the sectioning process. Evidence for mechanical deformation of the tissues was found in the outer nuclear layer of some of the retinas. In these cases [see Figure 3-5, a-d, for example], the columns of photoreceptor nuclei appeared compressed when compared to other control retinas. Based on the evidence available, it would appear that mechanical deformation, while present in some retinas, was probably not a major contributing factor to the variability seen in the control retinal tissue.

Finally, inherent subject variability may have contributed to the differences seen in the retinal tissue. The following observations support this possibility: [1] retinas fixed by perfusion and those fixed by immersion showed similar degrees of histological variability and [2] the two retinas of a single animal were similar in appearance although they were processed independently.

In conclusion, evidence available at the light microscopic level suggests that a number of factors may have been responsible for the differences observed in the histology of the control retinas. In conducting the analyses of the histological tissues obtained in this experiment, it was assumed that these factors influenced the retinal histology of both the control and experimental groups and therefore should not have confounded the analyses of the data.

3.2.2.2 Effects of 1345 lux on retinal morphology. Light microscopic examination of the retinas obtained from animals exposed to 1345 lux for 8 or 29 days revealed gross abnormalities in the retinal layers containing the photoreceptor outer and inner segments and photoreceptor nuclei (Figure 3-6). Following 8 or 29 days exposure to 1345 lux, the most obvious light-induced histological changes were seen in the SON region of the retina (Figure 3-6, b,f). In this region, the loss of photoreceptor nuclei from the outer nuclear layer was quite dramatic and often only one row of nuclei remained in the retinas of animals exposed to 1345 lux for 29 days. Very few photoreceptor outer or inner segments were present in these retinas and macrophages were often found among the outer and inner segments of degenerating photoreceptor cells (Figure 3-6, b,c). The remaining three regions of the retina (SUP, ION and INF) showed similar signs of light damage to the photoreceptor cells although damage in these areas was less severe than that found in the SON region. in general, the most peripheral regions of the retina (Figure 3-6, a,d,e,h) showed the least amount of damage.

Figure 3-6. Light photomicrographs of retinal tissue taken from two experimental animals exposed to 1345 lux for either 8 days (panels a-d) or 29 days (panels e-h). Panels a and e were taken of the SUP retinal region, b and f were taken of the SON retinal region, c and g were taken of the ION retinal region and d and h were taken of the INF retinal region. Both 8 and 29 days exposure to 1345 lux produced severe retinal damage which was characterized by loss of photoreceptor cells (thinning of OS and ONL layers). Macrophages (panels b and c, arrows) were occasionally observed in the retinal layer containing the degenerating photoreceptor outer segments. Damage was most severe in the SON region where often only one row of photoreceptor nuclei could be found (panel f, arrow showing location of ONL). Abbreviations: OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer. Calibration bar = 150 µm.


OS
ONL
INL
a
b
c
d
e
f
g
h

Morphometric analyses of the retinas of animals exposed to 1345 lux for 8 or 29 days were carried out to determine the effect of experimental light exposure on the widths of the various layers of the retina. The results of these analyses showed that a significant reduction in the widths of the retinal layers containing the photoreceptor outer and inner segments and photoreceptor nuclei occurred in the SON region following 8 days exposure. Similar changes were observed in the remaining three regions of the retina after 29 days exposure.

The mean widths obtained for the five retinal layers measured in each of the four regions of the retinas examined are shown in Figure 3-7. Mean values calculated for the control and experimental groups were compared within each of the four retinal regions using 20 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at p=0.05. The results of the between group comparisons of the widths of the five retinal layers showed that there was a significant between groups effect for the outer nuclear layer and the retinal layer containing the photoreceptor outer and inner segments in all four retinal regions examined and for the inner plexiform layer in the ION retinal region (see Table 3-3 for ANOVA results). Post-hoc tests on mean widths of the layer containing the photoreceptor outer and inner segments determined for the SUP, SON and ION regions of the retina revealed that the values calculated for animals exposed to

Figure 3.7. Results of morphometric analyses of retinas of animals exposed to 1345 lux for 8 or 29 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). The widths of five retinal layers (um) were determined in each of four retinal regions (A = SUP, B = SON, C = ION and D = INF). Each bar represents the group mean (+SE). Experimental group means marked with an asterisk (*) were significantly different (p=0.05) from the corresponding control group means. Abbreviations: PE - pigment epithelium; ONL - outer nuclear layer; OS+IS - photoreceptor outer plus inner segments; INL - inner nuclear layer; IPL - inner plexiform layer.

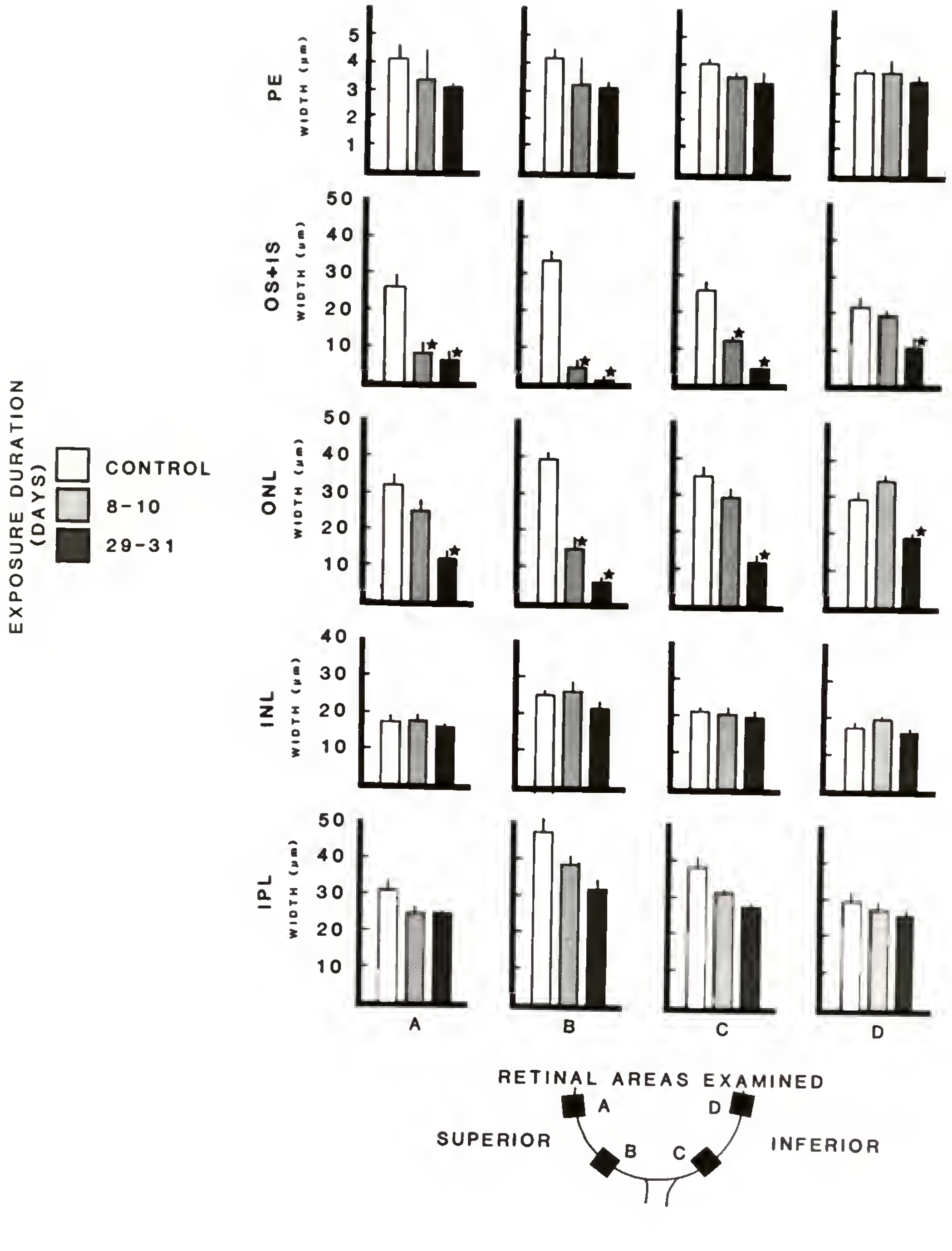

EXPOSURE DURATION (DAYS)
CONTROL
8-10
29-31
PE
WIDTH (µm)
1
2
3
4
5
OS+IS
WIDTH (µm)
10
20
30
40
50
ONL
WIDTH (µm)
10
20
30
40
50
INL
WIDTH (µm)
10
20
30
40
IPL
WIDTH (µm)
10
20
30
40
50
A
B
C
D
RETINAL AREAS EXAMINED
A
B
C
D
SUPERIOR
INFERIOR

TABLE 3-3

QUANTITATIVE ANALYSES OF MORPHOLOGY: COMPARISONS OF MEAN RETINAL LAYER WIDTHS OBTAINED FROM CONTROL AND EXPERIMENTAL ANIMALS EXPOSED TO 1345, 270, 130 OR 65 LUX ACROSS THE VARIOUS DAYS OF EXPOSURE TESTED AND WITHIN THE FOUR RETINAL REGIONS EXAMINED USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

LIGHT INTENSITY	RETINAL LAYERS		RETINAL REGION			
			SUP	SON	ION	INF
		(df)	(2,14)	(2,14)	(2,14)	(2,14)
1345 lux	PE		0.85[†]	1.73	3.65	0.35
	OS + IS		15.12 ***	34.92 ***	33.45 ***	4.52 *
	ONL		10.28 **	63.45 ***	27.03 ***	8.35 **
	INL		0.45	2.02	0.43	1.44
	IPL		2.11	3.51	3.89 *	0.46
		(df)	(6,36)	(6,36)	(6,36)	(6,35)
270 lux	PE		1.39	2.05	1.29	1.16
	OS + IS		5.34 ***	5.23 ***	4.96 ***	3.67 **
	ONL		5.85 ***	7.45 ***	6.79 ***	7.95 ***
	INL		1.05	1.55	2.18	3.07 *
	IPL		1.97	1.47	2.51 *	2.15
		(df)	(7,40)	(7,41)	(7,41)	(7,40)
130 lux	PE		1.27	2.50 *	2.05	4.03 **
	OS + IS		4.18 **	1.41	.90	2.67 *
	ONL		2.78 *	3.47 **	2.67 *	3.18 **
	INL		2.21	3.10 *	3.77 **	4.97 ***
	IPL		4.76 ***	2.28 *	3.96 **	5.35 ***
		(df)	(7,38)	(7,39)	(7,39)	(7,38)
65 lux	PE		.60	.73	.74	.99
	OS + IS		2.59 *	1.22	1.03	1.89
	ONL		.57	.49	1.70	1.53
	INL		1.24	1.05	2.78 *	1.75
	IPL		3.29 **	4.93 ***	7.58 ***	3.06 *

[†] ANOVA F value, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

1345 lux for 8 or 29 days were significantly smaller than the corresponding control values. In the INF region of the retina, the mean width of the layer containing the photoreceptor outer and inner segments determined for animals exposed to 1345 lux for 29 days was significantly smaller than the mean control value. The mean width of this layer determined for animals exposed to 1345 lux for 8 days did not differ from the mean control value in this region. Post-hoc tests on mean outer nuclear layer widths determined for the SUP, ION and INF regions of the retina revealed that the values calculated for animals exposed to 1345 lux for 29 days were significantly smaller than the corresponding control values. The mean outer nuclear layer widths determined for animals exposed to 1345 lux for 8 days were not different from the corresponding mean control values in these regions. In the SON region of the retina, the mean outer nuclear layer widths determined for animals exposed to 1345 lux for 8 or 29 days were significantly smaller than the corresponding control value. Finally, post-hoc tests on mean inner plexiform layer widths determined for the ION region of the retina revealed that the values calculated for animals exposed to 1345 lux for 8 or 29 days were not different from the corresponding control value.

The results obtained from the semi-quantitative analyses of the retinas of animals exposed to 1345 lux for 8 or 29 days confirmed the morphometric data. Mean total

TABLE 3-4

SEMI-QUANTITATIVE ANALYSES OF MORPHOLOGY: MEAN TOTAL SCORES [+SD] FOR THE HISTOLOGICAL CHANGES OBSERVED IN THE FOUR REGIONS OF THE RETINA ANAYLYZED FOLLOWING EXPOSURE TO 1345, 270, 130 OR 65 LUX

LIGHT INTENSITY	DAYS EXPOSURE	RETINAL REGION			
		SUP	SON	ION	INF
CONTROL		4.7 [0.8]	5.4 [1.9]	5.3 [1.6]	5.4 [1.4]
1345 lux	8	13.7 [1.5]	14.8 [3.9]	13.1 [1.4]	10.5 [3.6]
	29	15.4 [1.4]	17.0 [0.5]	15.5 [1.1]	13.1 [2.8]
270 lux	1	5.8 [1.1]	5.5 [1.9]	5.3 [2.5]	4.4 [0.5]
	3	4.7 [2.6]	8.5 [4.1]	6.6 [2.4]	4.8 [0.6]
	7	8.1 [2.9]	10.4 [5.0]	6.7 [3.5]	7.2 [3.3]
	14	8.7 [3.5]	11.8 [4.0]	9.7 [3.6]	7.7 [3.7]
	28	7.9 [3.5]	13.5 [3.4]	9.3 [4.1]	6.9 [2.9]
	59	12.0 [4.8]	14.8 [3.9]	14.3 [3.8]	14.7 [1.4]
130 lux	1	6.5 [2.8]	6.4 [1.9]	6.2 [2.9]	6.2 [1.9]
	3	5.5 [2.6]	6.7 [2.6]	6.6 [3.4]	7.3 [2.1]
	7	6.3 [2.4]	7.4 [3.1]	6.2 [2.9]	6.3 [2.3]
	14	7.5 [3.1]	7.1 [2.2]	6.0 [2.7]	5.4 [1.9]
	28	5.2 [2.3]	6.4 [1.8]	6.3 [1.9]	6.5 [1.5]
	59	8.0 [1.7]	7.8 [3.1]	5.6 [2.9]	6.6 [1.6]
	85	7.0 [2.5]	7.7 [2.7]	6.2 [2.9]	6.5 [2.1]
65 lux	1	6.2 [1.9]	5.6 [1.1]	4.2 [2.8]	5.1 [2.5]
	3	6.1 [1.3]	5.3 [1.6]	5.4 [2.9]	6.2 [2.0]
	7	5.6 [1.6]	5.3 [2.2]	4.6 [2.4]	5.5 [1.9]
	14	5.6 [1.4]	4.6 [1.6]	5.0 [3.0]	5.5 [2.1]
	28	6.3 [1.6]	5.6 [1.8]	6.3 [2.9]	4.5 [2.0]
	59	7.3 [1.8]	6.2 [2.6]	6.3 [2.9]	6.8 [1.8]
	85	7.0 [1.3]	6.0 [2.6]	5.8 [3.2]	5.9 [2.6]

histological scores obtained for each of the four retinal regions examined in each group ranged from 10.5 to 17 and are shown in Table 3-4. Possible scores ranged from 5 to 18 with a score of 18 being given to retinas with severe light damage. These scores were compared to the corresponding control scores using t-tests (significance level set at p=0.05). The results of these comparisons, which are shown in Table 3-5, revealed that the scores obtained for animals exposed to 1345 lux for 8 or 29 days were significantly greater than the corresponding control scores in all four retinal regions examined.

3.2.2.3 Effects of 270 lux on retinal morphology. Light microscopic examination of the retinas obtained from animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days revealed that changes in retinal morphology due to light damage could be observed in retinas of animals exposed to 270 lux for as little as 1 day. Damage observed within a group of animals often appeared to fall into two categories; therefore, examples taken from each category will be shown. Figures 3-8 and 3-9 show the least and greatest amounts of damage to the SON regions of retinas of animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days.

The SON regions of retinas taken from animals exposed to 270 lux for 1 day are shown in Figure 3-8, a and d. Histology taken from a retina with the greatest amount of damage (Figure 3-8d) revealed that pyknotic photoreceptor nuclei were present in the outer nuclear layer and that the

TABLE 3-5

SEMI-QUANTITATIVE ANALYSES OF MORPHOLOGY: COMPARISONS OF MEAN TOTAL HISTOLOGICAL RETINAL SCORES OBTAINED FROM CONTROL AND EXPERIMENTAL ANIMALS EXPOSED TO 1345, 270, 130 OR 65 LUX BY RETINAL REGION USING T-TESTS

LIGHT INTENSITY	DAYS EXPOSURE	(n)	RETINAL REGION SUP	SON	ION	INF
1345 lux	8	8	14.10† ***	5.73 **	13.56 ***	3.90 **
	29	8	20.35 ***	25.42 ***	20.59 ***	7.32 ***
270 lux	1	14	3.18 ***	.22	0.00	3.14 ***
	3	10	0.00	2.07 *	1.48	1.66
	7	12	3.91 ***	3.20 ***	1.29	1.85 *
	14	12	3.93 ***	5.05 ***	4.03 ***	1.46
	28	10	2.53 **	7.12 ***	3.03 **	1.53
	59	6	3.74 **	5.80 ***	5.74 ***	14.51 ***
130 lux	1	12	2.74 **	1.43	.98	1.23
	3	12	.96	1.55	1.26	2.76 **
	7	12	2.33 **	2.12 *	.98	1.12
	14	12	3.09 **	2.25 **	.84	.02
	28	10	.68	1.47	1.49	1.92 *
	59	12	6.35 ***	2.33 **	.32	1.89 *
	85	10	2.81 **	2.46 **	.94	1.34
65 lux	1	6	1.82	.37	.95	.31
	3	12	3.34 ***	.12	.15	1.19
	7	10	1.71	.02	.84	.13
	14	8	1.76	1.03	.26	.11
	28	12	3.40 ***	.35	1.17	1.29
	59	12	4.76 ***	.95	1.06	2.20 *
	85	11	5.26 ***	.75	.47	.60

* $p<.05$ (one-tailed), ** $p<.025$, *** $p<.005$

† Unpaired 't'-value computed for unequal variances (Darlington, 1975)

Figure 3-8. Light photomicrographs of retinal tissue taken from animals exposed to 270 lux. Panels a-c were taken of retinas of animals exposed to 270 lux for 1, 3 or 7 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels d-f are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. Vesiculated apical OS membrane (panel d, open arrow) and pyknotic nuclei (panel d, filled arrows and inset) were observed in 50% of the retinas exposed to 270 lux for 1 day. Macrophages were often present in the damaged outer segment layer (open arrows, panel e). Least damaged cases showed little evidence of light damage. Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; IS - photoreceptor inner segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer. Calibration bar = 150 µm.

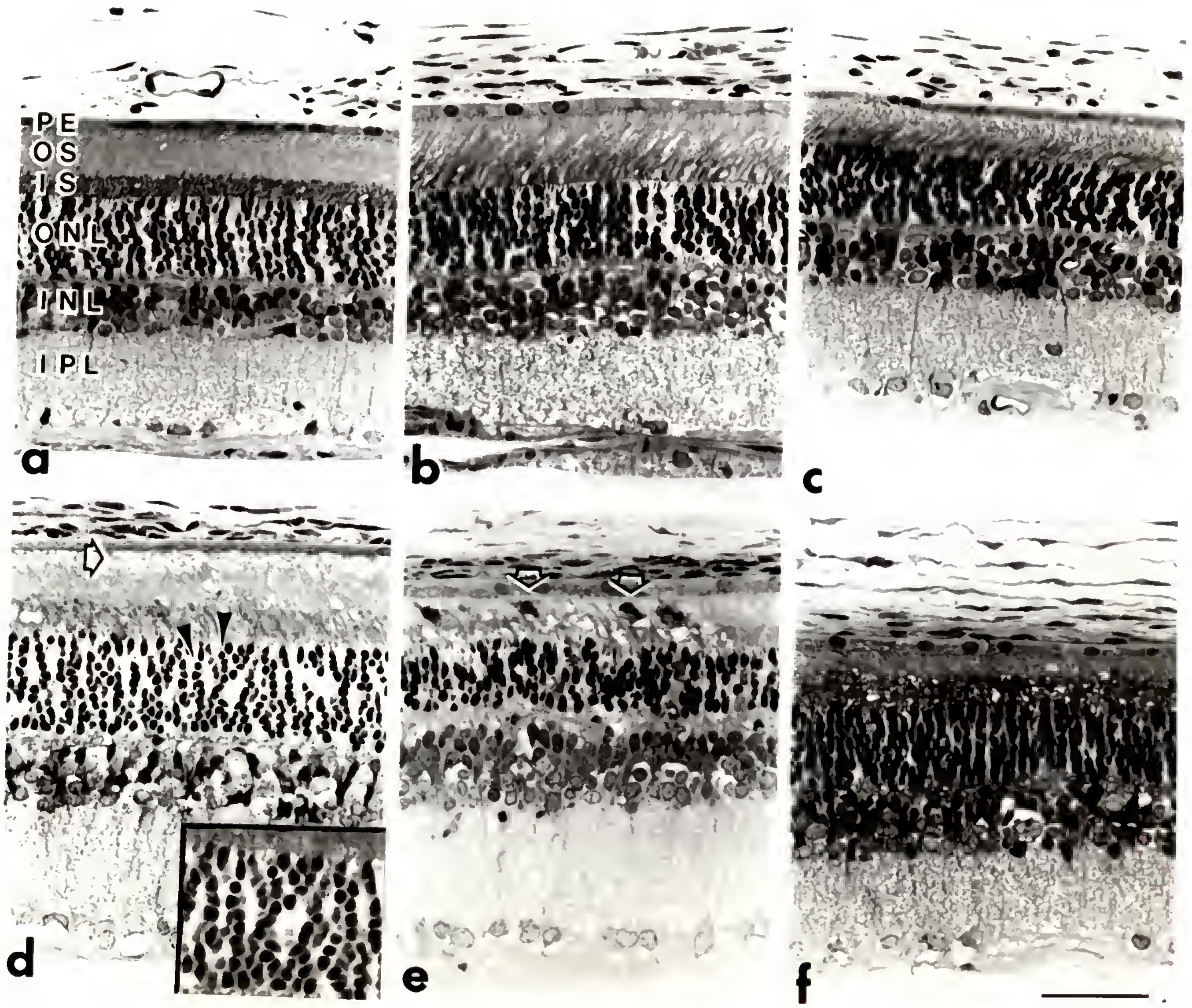
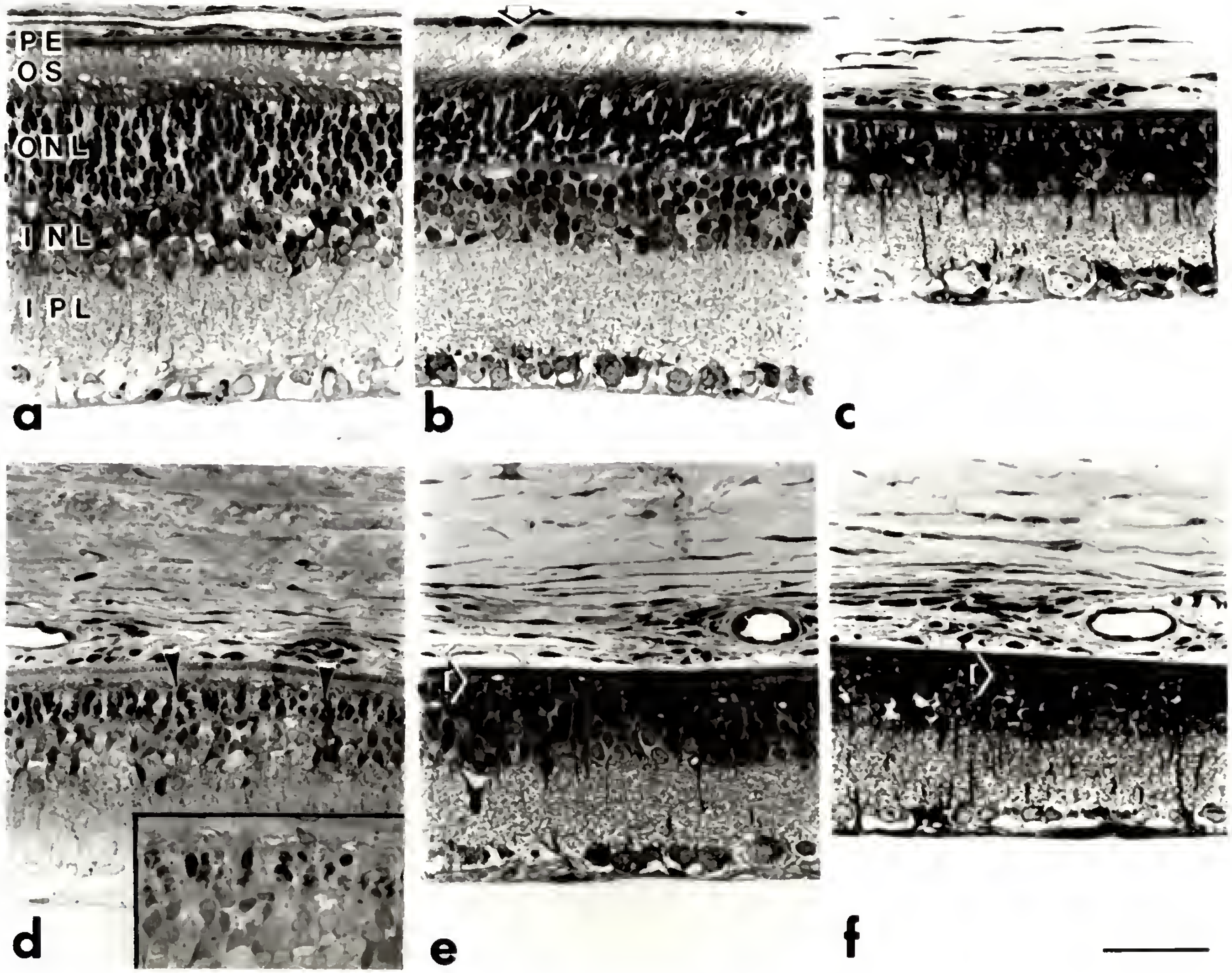

PE
OS
IS
ONL
INL
IPL
a
b
c
d
e
f

Figure 3-9. Light photomicrographs of retinal tissue taken from animals exposed to 270 lux. Panels a-c were taken of retinas of animals exposed to 270 lux for 14, 28 or 59 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels d-f are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. Least damaged retinas began to show evidence of light damage by 14 days exposure (note vesiculated OS membranes, panel a). Macrophages were present in the damaged outer segment layer of these retinas by 28 days exposure (panel b, open arrow). Most of the nuclei in the ONL were pyknotic in the severely damaged retinas and the majority of those which appeared normal were the cone nuclei (closed arrows, panel d and inset). Severely damaged retinas contained one row of photoreceptor nuclei in the ONL (open arrows, panels e and f). Note the substantial reduction in the width of the outer nuclear layer with increasing exposure duration (panels a-c). Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer. Calibration bar = 150 μm.


PE
OS
ONL
INL
IPL
a
b
c
d
e
f

apical tips of the photoreceptor outer segments appeared vesiculated. These changes, which are characteristic of mild light damage, were seen in 50% (3 out of 6 cases) of the retinas taken from this group of animals. The retina shown in Figure 3-8a, which provides an example of the other 50% of the cases, does not show evidence of light damage at the light microscopic level.

The SON regions of retinas taken from animals exposed to 270 lux for 3 days are shown in Figure 3-8, b and e. In the cases of greatest damage (60%, 3 out of 5 cases), three days exposure to 270 lux appeared to be sufficient to cause nearly a 50% reduction in the number of nuclei in the outer nuclear layer (Figure 3-8e). A substantial number of the remaining nuclei in this layer appeared pyknotic. Macrophages were often present in the retinal layers which contained degenerating photoreceptor inner and outer segments. The retina shown in Figure 3-8b, which is representative of 40% of the cases (2 out of 5), does not show evidence of light damage at the light microscopic level.

The SON regions of retinas taken from animals exposed to 270 lux for 7 days are shown in Figure 3-8, c and f. The appearance of these retinas was similar to those taken from animals exposed to 270 lux for 3 days. The appearance of the retinas of 33% of the animals examined from this group (2 out of 6 cases) resembled that shown in Figure 3-8c with the least damage. The remaining animals' retinas (4 out of 6 cases) resembled that shown in Figure 3-8f.

The SON regions of retinas taken from animals exposed to 270 lux for 14 days are shown in Figure 3-9, a and d. Examination of histology taken from the most severely damaged retinas (50%, 3 out of 6 cases) (Figure 3-9d) revealed that the outer nuclear layer consisted of 2-3 rows of photoreceptor nuclei. Many of the nuclei were pyknotic and those which appeared normal were primarily cone nuclei. The layers of the retina containing the photoreceptor inner and outer segments were nearly gone at this stage. Figure 3-9a shows the SON region of a retina showing the least amount of damage incurred from exposure to 270 lux for 14 days and is representative of 50% of the cases examined in this group (3 out of 6 cases). The main indication for light damage in this particular example was the presence of vesicles in the apical region of the photoreceptor outer segments. The number of nuclei in the outer nuclear layer was not lower than control levels.

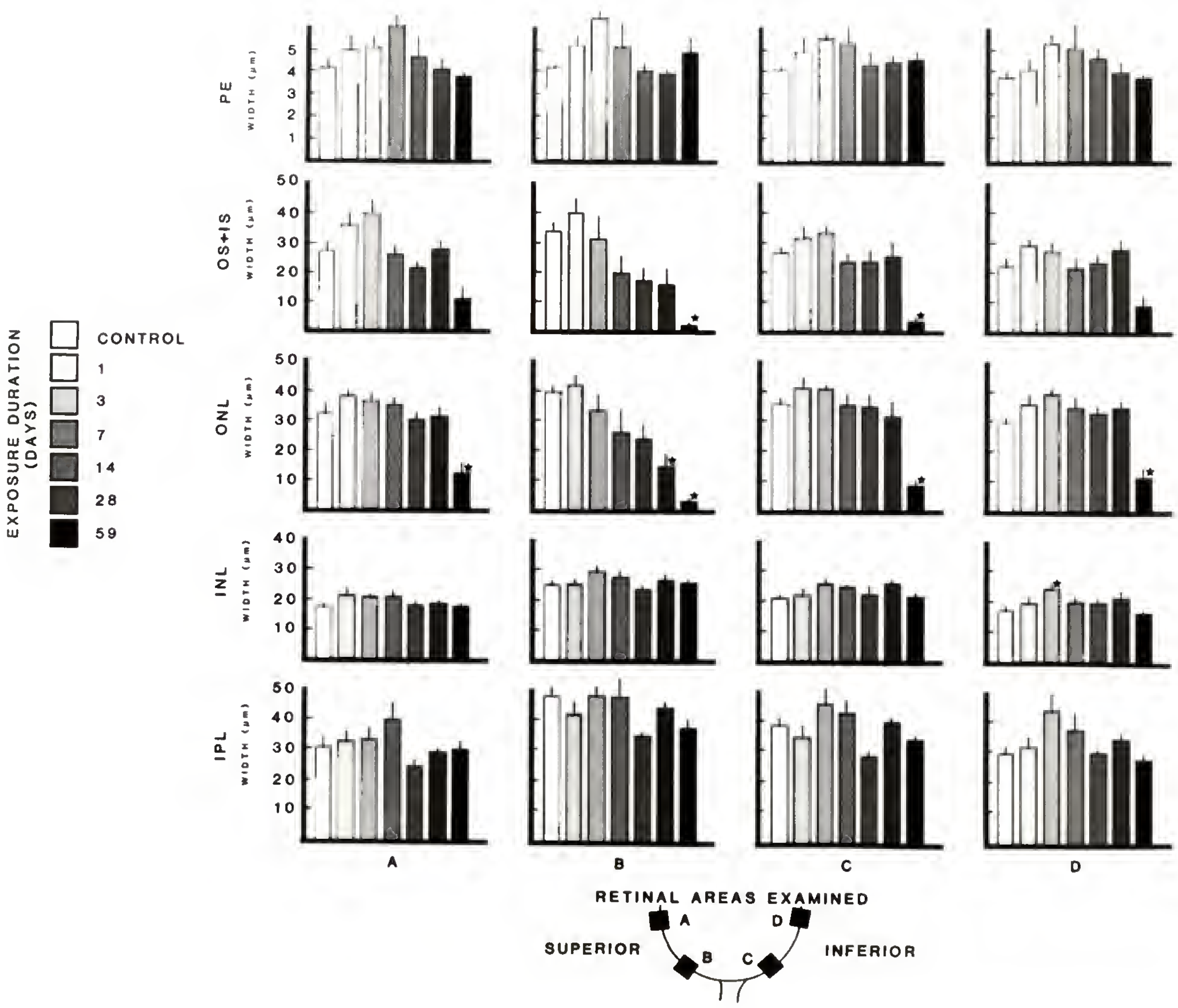
The SON regions of retinas taken from animals exposed to 270 lux for 28 days are shown in Figure 3-9, b and e. In the most severely damaged cases (80%, 4 out of 5 cases) (Figure 3-9e), the nuclei in the outer nuclear layer had been reduced to a single row and the outer limiting membrane, which usually separates the outer nuclear layer from the photoreceptor inner segments, was adjacent to the cells of the pigment epithelium. The SON region of the least damaged retina taken from an animal exposed to 270 lux for 28 days is shown in Figure 3-9b. The presence of macrophages in the retinal layer containing the photoreceptor outer segments indicated that this retina was damaged. There also appeared to be some loss of nuclei from the outer nuclear layer.

Finally, the SON regions of retinas taken from animals exposed to 270 lux for 59 days are shown in Figure 3-9, c and f. Examination of the retinal histology obtained from this group of animals revealed that all of the retinas had been severely damaged (100%, 3 out of 3 cases). In the cases shown, the outer nuclear layer was reduced to 1 row of photoreceptor nuclei and the retinal layers containing the photoreceptor inner and outer segments were nonexistent. The remaining retinal layers appeared fairly normal.

Morphometric analyses of the retinas of animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days were carried out to determine the effect of experimental light exposure on the widths of the various layers of the retina. In general, the results of these analyses showed that there was a gradual reduction in the widths of the retinal layers containing the photoreceptor cells in all regions examined over the course of the 59 day exposure period.

The mean widths obtained for the five retinal layers measured in each of the four regions of the retinas examined are shown in Figure 3-10. Mean values calculated for the control and experimental groups were compared within each of

Figure 3-10. Results of morphometric analyses of retinas of animals exposed to 270 lux for 1, 3, 7, 14, 28, or 59 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). The widths of five retinal layers (um) were determined in each of four retinal regions (A = SUP, B = SON, C = ION and D = INF). Each bar represents the group mean (+SE). Experimental group means marked with an asterisk (*) were significantly different ($p=0.05$) from the corresponding control group means. Abbreviations: PE - pigment epithelium; ONL - outer nuclear layer; OS+IS - photoreceptor outer plus inner segments; INL - inner nuclear layer; IPL - inner plexiform layer.


EXPOSURE DURATION (DAYS)
CONTROL
1
3
7
14
28
59
PE
WIDTH (µm)
5
4
3
2
1
OS+IS
WIDTH (µm)
50
40
30
20
10
ONL
WIDTH (µm)
50
40
30
20
10
INL
WIDTH (µm)
40
30
20
10
IPL
WIDTH (µm)
50
40
30
20
10
*
A
B
C
D
RETINAL AREAS EXAMINED
SUPERIOR
INFERIOR
A
B
C
D

the four retinal regions using 20 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at p=0.05. The results of the between group comparisons of the widths of the five retinal layers showed that there was a significant between groups effect for the retinal layer containing the photoreceptor inner and outer segments and the outer nuclear layer in all four retinal regions examined. A significant between groups effect was also obtained for the inner plexiform layer in the ION retinal region and for the inner nuclear layer in the INF retinal region (see Table 3-3 for ANOVA results). Post-hoc tests on mean widths of the retinal layer containing the photoreceptor inner and outer segments determined for the SON and ION regions of the retina revealed that the values calculated for animals exposed to 270 lux for 59 days were significantly smaller than the corresponding control values. The mean widths of the retinal layer containing the photoreceptor inner and outer segments determined for animals exposed to 270 lux for 1, 3, 7, 14 or 28 days were not different from the corresponding mean control values in these regions. Post-hoc tests on mean widths of the retinal layer containing the photoreceptor inner and outer segments determined for the SUP and INF regions of the retina revealed that the values calculated for animals exposed to 270 lux were not different from the corresponding control values. Post-hoc tests on mean outer nuclear layer widths determined for the SUP, ION and INF regions of the retina revealed that the values calculated for animals exposed to 270 lux for 59 days were significantly smaller than the corresponding control values. The mean outer nuclear layer widths determined for animals exposed to 270 lux for 1, 3, 7, 14 or 28 days were not different from the corresponding mean control values in these regions. In the SON region of the retina, the mean outer nuclear layer widths determined for animals exposed to 270 lux for 28 or 59 days were significantly smaller than the corresponding control value. The mean outer nuclear layer widths calculated for animals exposed to 270 lux for 1, 3, 7 or 14 days were not different from the control value in the SON region of the retina. Post-hoc tests on mean inner nuclear layer widths determined for the INF region of the retina revealed that the value calculated for animals exposed to 270 lux for 3 days was significantly greater than the corresponding control value. The mean inner nuclear layer widths calculated for animals exposed to 270 lux for 1, 7, 14, 28 or 59 days were not different from the control value. Finally, post-hoc tests on mean inner plexiform layer widths determined for the ION region of the retina revealed no difference from controls.

The results obtained from semi-quantitative comparisons of the retinas of animals exposed to 270 lux for 1, 3, 7, 14, 28 or 59 days with the retinas of controls suggest that the pigment epithelium and the retinal layers containing the photoreceptor nuclei and inner and outer segments of animals exposed to 270 lux may be significantly altered by as few as 1 to 3 days exposure. Mean total histological scores obtained for each of the groups in the four retinal regions examined are shown in Table 3-4. In general, the mean scores in each region increased as the animals were exposed to 270 lux for longer periods of time. These scores were compared to the corresponding control scores using t-tests with the significance level set at $p=0.05$. The results of these comparisons, which are shown in Table 3-5, revealed that mean scores for the SUP retinal regions of animals exposed to 270 lux were significantly greater than the corresponding mean control scores at all times except 3 days exposure. Mean scores obtained for the SON retinal regions were significantly greater than the corresponding mean control score for all times except following 1 day exposure. The mean scores obtained for the ION retinal regions of animals exposed to 270 lux for 14, 28 or 59 days were significantly greater than the corresponding mean control score. Finally, the mean scores for the INF retinal regions of animals exposed to 270 lux for 7 or 59 days were significantly greater than the corresponding control score. The mean score obtained for animals exposed to 270 lux for 1 day was significantly less than that obtained for the control group in this region.

3.2.2.4 Effects of 130 lux on retinal morphology. Light microscopic examination of the retinas obtained from animals exposed to 130 lux for 1, 3, 7, 14, 28, 59 or 85

Figure 3-11. Light photomicrographs of retinal tissue taken from animals exposed to 130 lux. Panels a-d were taken of retinas of animals exposed to 130 lux for 1, 3, 7 or 14 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels e-h are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. Mild light damage, indicated by vesiculated outer segment membranes and swollen photoreceptor inner segments (closed arrow, panel f), was observed by 3 days exposure in 33% of the cases examined. Pyknotic nuclei were observed in the outer nuclear layer of the retinas of 33% of the animals exposed to 130 lux for 14 days (closed arrows, panel h). Least damaged retinas showed little evidence of light damage (panels a-d). Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer. Calibration bar = 150 µm.

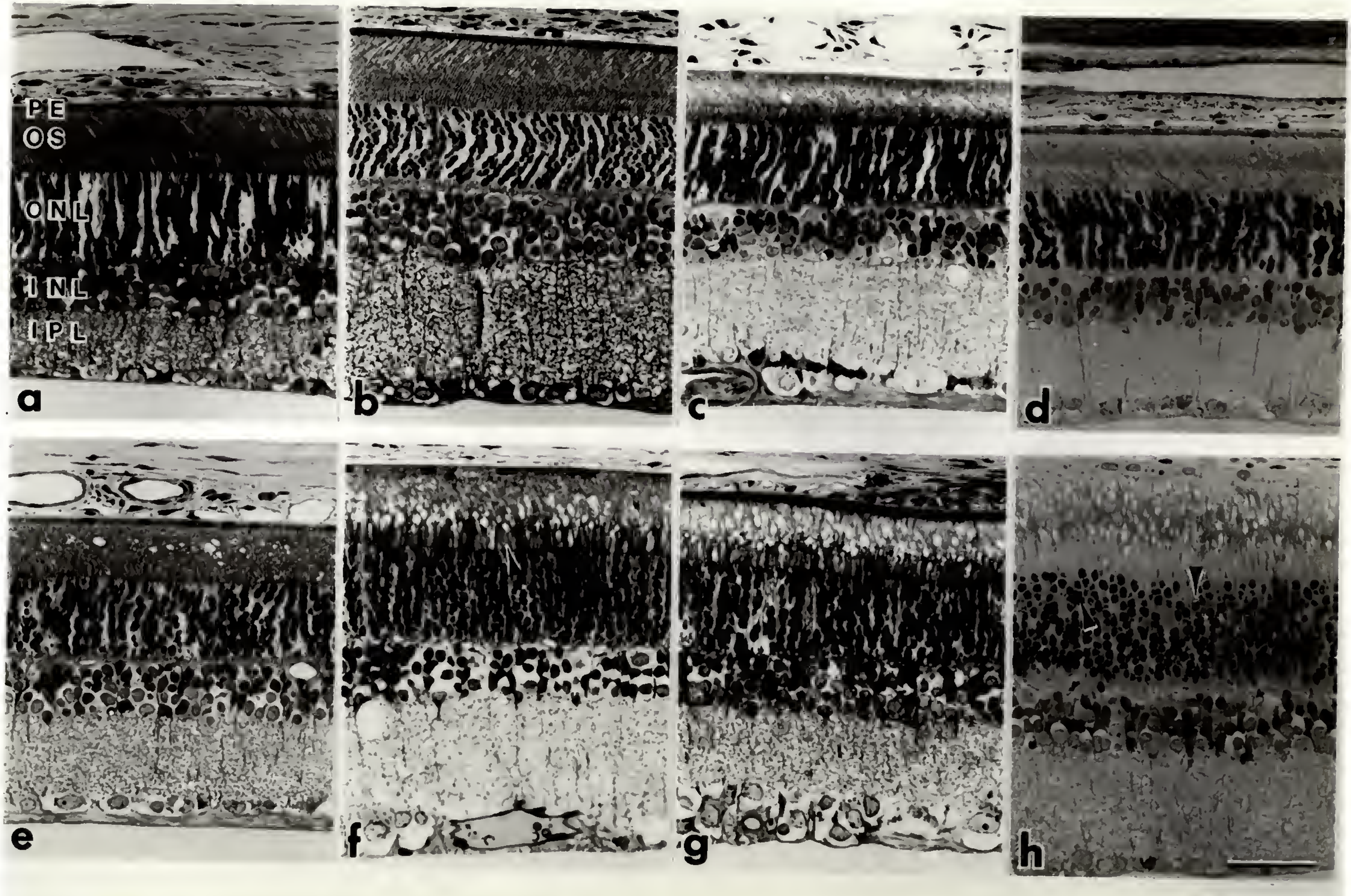
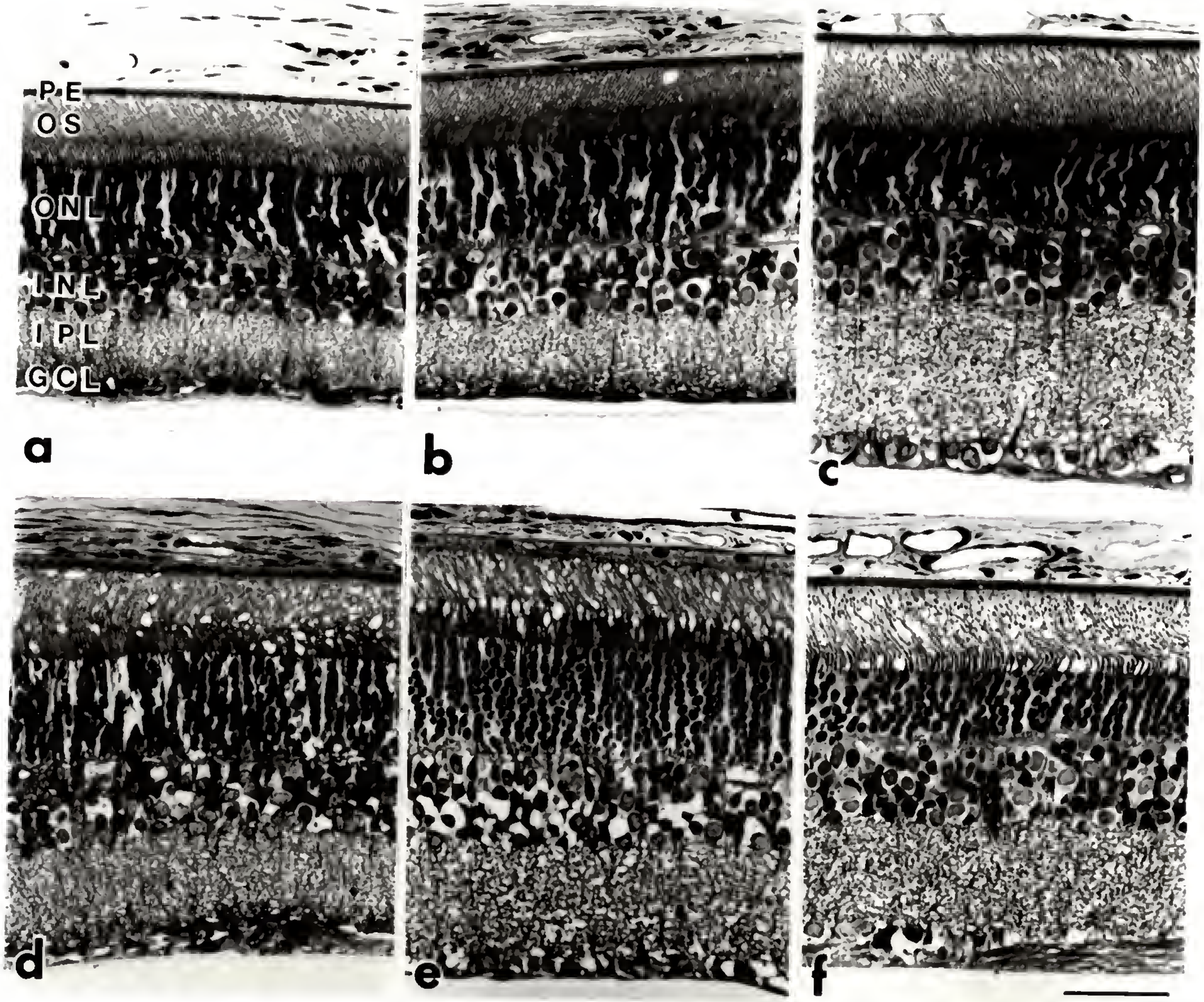

PE
OS
ONL
INL
IPL
a
b
c
d
e
f
g
h

Figure 3-12. Light photomicrographs of retinal tissue taken from animals exposed to 130 lux. Panels a-c were taken of retinas of animals exposed to 130 lux for 28, 59 or 85 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels d-f are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. Vesiculated outer segments (panels d, e and f), swollen inner segments (panel e) and loss of photoreceptor nuclei from the outer nuclear layer (panel f) were evident in the most severely damaged retinas. The majority of the cases examined (60% or greater) did not show evidence of light damage (panels a-c). Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer; GCL - ganglion cell layer. Calibration bar = 150 µm.


P.E
OS
ONL
INL
IPL
GCL
a
b
c
d
e
f

days revealed that the primary change in the morphology of these retinas occurred in the layers of the retina containing the outer segments, inner segments and nuclei. In the groups of animals exposed to 130 lux for 3, 7, 14, 28, 59 or 85 days, the photoreceptor outer segments found in the retinas with the severest damage were often vesiculated and in some cases, pyknotic photoreceptor nuclei were present in outer nuclear layers of these retinas. Figures 3-11 and 3-12 show examples of the least and greatest amounts of damage observed in the SON regions of the retinas of these animals.

The SON regions of retinas taken from animals exposed to 130 lux for 1 day are shown in Figure 3-11, a and e. Examination of the retinal histology taken from this group of animals revealed little evidence of light damage. Scattered vesicles in the retinal layer containing the photoreceptor outer segments provided the only evidence that a few of the retinas may have been damaged by exposure to 130 lux for 1 day (33%, 2 out of 6 cases) (see Figure 3-11e).

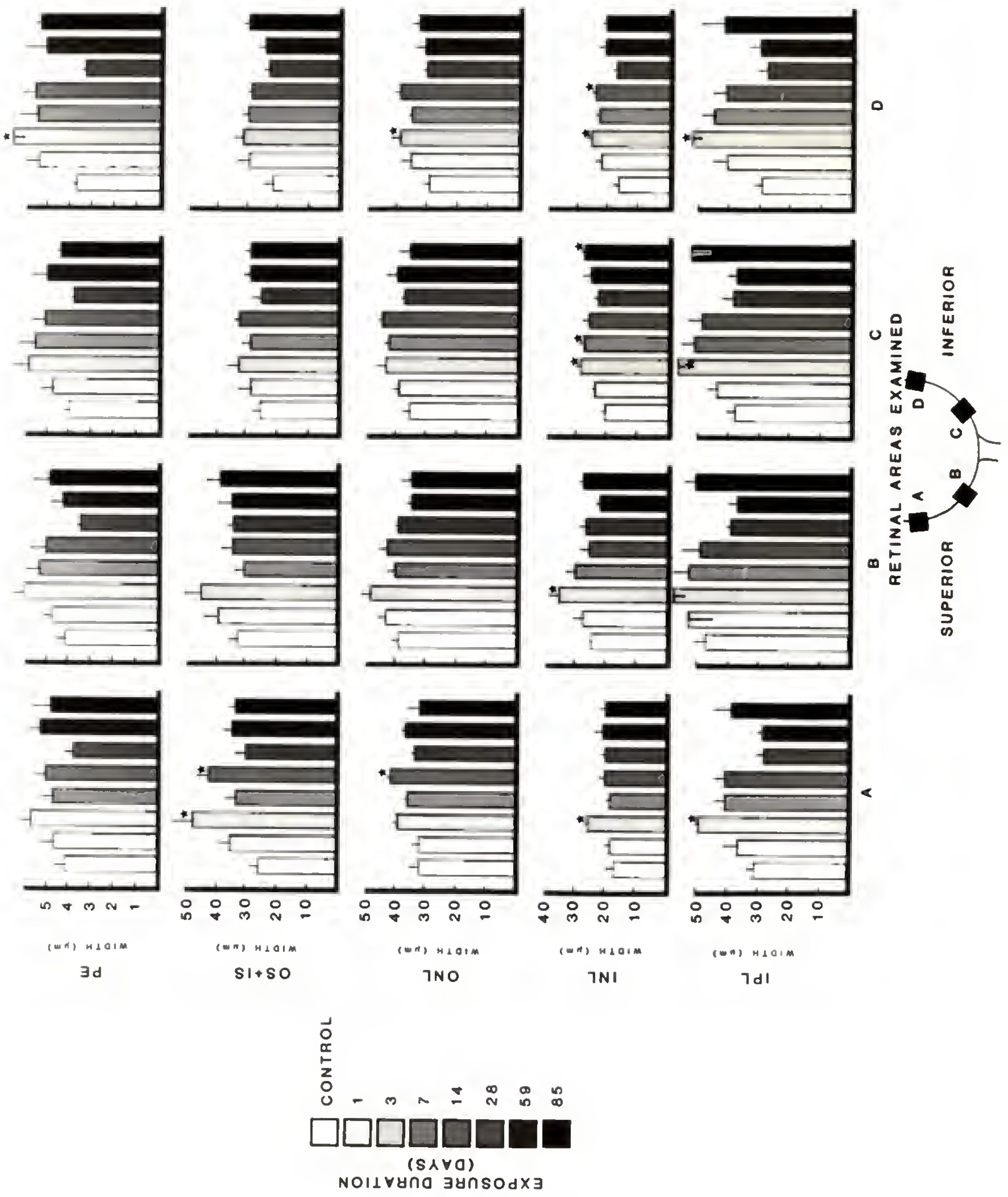
The SON regions of retinas taken from animals exposed to 130 lux for 3 or 7 days are shown in Figure 3-11, b and f, and Figure 3-11, c and g, respectively. In cases of greatest damage (Figure 3-11, f and g), the photoreceptor outer segments were vesiculated and some of the photoreceptor inner segments appeared swollen. These changes, which are characteristic of mild light damage, were seen in 33% (2 out of 6 cases for animals exposed for 3 days) and 66% (4 out of 6 cases for animals exposed for 7 days) of all retinas taken from these groups. Examples of the cases which did not show evidence of light damage at the light microscopic level are shown in Figure 3-11, b and c.

The SON regions of retinas taken from animals exposed to 130 lux for 14 days are shown in Figure 3-11, d and h. In cases of severest damage (33%, 2 out of 6 cases), photoreceptor outer segments were often vesiculated and pyknotic nuclei were found in the outer nuclear layers of retinas showing the greatest amount of damage (Figure 3-11h). Many of the retinas taken from this group of animals showed no evidence of light damage at the light microscopic level (66%, 4 out of 6 cases) (Figure 3-11d).

The SON regions of retinas taken from animals exposed to 130 lux for 28 or 59 days are shown in Figure 3-12, a and d, and 3-12, b and e, respectively. In cases of severest damage, the presence of vesiculated photoreceptor outer segment membranes provided the primary histological evidence for retinal damage in these animals (Figure 3-12, d and e). Many of the retinas taken from animals exposed to 130 lux for 28 or 59 days did not show evidence of light damage, 80% (4 out of 5 cases) and 60% (3 out of 5 cases), respectively (Figure 3-12, a and b).

Finally, the SON regions of retinas taken from animals exposed to 130 lux for 85 days are shown in Figure 3-12, c

Figure 3-13. Results of morphometric analyses of retinas of animals exposed to 130 lux for 1, 3, 7, 14, 28, 59 or 85 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). The widths of five retinal layers (um) were determined in each of four retinal regions (A = SUP, B = SON, C = ION and D = INF). Each bar represents the group mean (+ or - SE). Experimental group means marked with an asterisk (*) were significantly different ($p=0.05$) from the corresponding control group means. Abbreviations: PE - pigment epithelium; ONL - outer nuclear layer; OS+IS - photoreceptor outer plus inner segments; INL - inner nuclear layer; IPL - inner plexiform layer.


PE
OS+IS
ONL
INL
IPL
WIDTH (µm)
5
4
3
2
1
50
40
30
20
10
40
*
A
B
C
D
RETINAL AREAS EXAMINED
SUPERIOR
INFERIOR
EXPOSURE DURATION (DAYS)
CONTROL
1
3
7
14
28
59
85

and f. Examination of histology taken from retinas with severest damage revealed a loss of photoreceptor nuclei from the outer nuclear layer layer [Figure 3-12f]. The number of rows of nuclei in the outer nuclear layer of these retinas (40%, 2 out of 5 cases) was reduced from 8-10 to 6 and vesicles were present in the layer containing the photoreceptor outer segments. The SON region of least damaged retinas did not show evidence of light damage at the light microscopic level [Figure 3-12c].

Morphometric analyses of the retinas of animals exposed to 130 lux for 1, 3, 7, 14, 28, 59 or 85 days were carried out to determine the effect of experimental light exposure on the widths of the various layers of the retina. No significant reductions in the widths of the retinal layers were observed over the course of the 85 day test period. Some increases in the widths of a few of the retinal layers were observed.

The mean widths obtained for the five retinal layers measured in each of the four regions of the retinas examined are shown in Figure 3-13. Mean values calculated for the control and experimental groups were compared within each of the four retinal regions using 20 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at $p=0.05$. The results of between group comparisons of the widths of the five retinal layers showed that there was a significant between groups effect for the following retinal layers and regions: [1] the pigment epithelium in the SON and INF regions of the retina, (2) the layer of the retina containing the photoreceptor outer and inner segments in the SUP and INF regions of the retina, (3) the outer nuclear layer in all four regions of the retina examined, (4) the inner nuclear layer in the SON, ION and INF regions of the retina and (5) the inner plexiform layer in all four regions of the retina examined (see Table 3-3 for ANOVA results). The results of post-hoc tests revealed that whenever the experimental group values were significantly different from the corresponding control values, the experimental values were always larger than the control values.

Post-hoc tests on mean pigment epithelium widths determined for the INF regions of the retina revealed that the value calculated for animals exposed to 130 lux for 3 days was significantly larger than the corresponding control. In the SON region of the retina, the mean pigment epithelium widths determined for all experimental animals exposed to 130 lux were not different from the corresponding control value. Post-hoc tests on the mean widths of the retinal layer containing the photoreceptor outer and inner segments determined for the SUP region of the retina revealed that values calculated for animals exposed to 130 lux for 3 or 14 days were significantly larger than the corresponding control values. In the INF region of the retina, the mean widths of the retinal layer containing the photoreceptor outer and inner segments determined for all experimental animals exposed to 130 lux were not different from the corresponding control value. Post-hoc tests on mean outer nuclear layer widths calculated for the SUP region of the retina revealed that the value determined for animals exposed to 130 lux for 14 days was significantly larger than the corresponding control value. Post-hoc tests on mean outer nuclear layer widths calculated for the INF region of the retina revealed that the value determined for animals exposed to 130 lux for 3 days was significantly larger than the corresponding control value. In the SON and ION regions of the retina, the mean outer nuclear layer widths determined for all experimental animals exposed to 130 lux were not different from the corresponding control values. Post-hoc tests on mean inner nuclear layer widths calculated for all four regions of the retina revealed that the value determined for animals exposed to 130 lux for 3 days was significantly larger than the corresponding control values. In addition, the mean inner nuclear layer widths determined for the ION regions of retinas of animals exposed to 130 lux for 7 or 85 days and the mean inner nuclear layer width determined for the INF region of retinas of animals exposed to 130 lux for 14 days were significantly larger than the corresponding control values. Finally, post-hoc tests on mean inner plexiform layer widths determined for the SUP, ION and INF regions of the retina revealed that the values calculated for animals exposed to 130 lux for 3 days in each region were significantly larger than the corresponding control values. The mean inner plexiform layer widths determined for the SON region of retinas of experimental animals exposed to 130 lux were not different from the mean control value.

The results obtained from semi-quantitative comparisons of the retinas of animals exposed to 130 lux for 1, 3, 7, 14, 28, 59 or 85 days with those of control animals revealed that differences could be observed in the pigment epithelium, the layer of the retina containing the photoreceptor outer and inner segments and the outer nuclear layer of the retinas of animals exposed to 130 lux; however, there was no discernable pattern to the observed differences with respect to the duration of exposure to 130 lux. Mean total histological scores obtained for each of the groups in the four retinal regions examined are shown in Table 3-4. These scores were compared to the corresponding control scores using t-tests with the significance level set at p=0.05.

The results of these comparisons, which are shown in Table 3-5, revealed that mean scores for the SUP regions of retinas of animals exposed to 130 lux for 1, 7, 14, 59 and 85 days were significantly greater than the corresponding mean control score. Mean scores obtained for the SON regions of retinas of animals exposed to 130 lux for 7, 14, 59 or 85 days were significantly greater than the corresponding mean control value. Finally, mean scores obtained for the INF regions of retinas of animals exposed to 130 lux for 3, 28 or 59 days were significantly greater than the corresponding mean control value. The mean scores obtained for the ION regions were not different from the corresponding mean control value.

3.2.2.5 Effects of 65 lux on retinal morphology. Light microscopic examination of the retinas obtained from animals exposed to 65 lux for 1, 3, 7, 14, 28, 59 or 85 days revealed little difference between the histology of retinas showing the least and greatest amounts of damage. Figures 3-14 and 3-15 show examples of the least and greatest amounts of damage observed in the SON regions of these retinas.

The SON regions of retinas taken from animals exposed to 65 lux for 1, 3, 7, or 14 days are shown in Figure 3-14. In the cases of greatest damage (Figure 3-14, e-h), scattered vesicles were present in the retinal layer containing the photoreceptor outer segments. No pyknotic photoreceptor nuclei were observed in the outer nuclear layers of these retinas. The most severely damaged retinas in each group accounted for 25% or fewer of the cases examined. The least damaged retinas in these groups (Figure 3-14, a-d) did not show any evidence of light damage.

The SON regions of retinas taken from animals exposed to 65 lux for 28 or 59 days are shown in Figure 3-15 a, b, d and e. After 28 or 59 days of exposure to 65 lux, scattered vesicles were observed in the photoreceptor outer segment layers of the most severely damaged retinas (Figure 3-15, d

Figure 3-14. Light photomicrographs of retinal tissue taken from animals exposed to 65 lux. Panels a-d were taken of retinas of animals exposed to 65 lux for 1, 3, 7 or 14 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels e-h are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. Scattered vesicles in the OS layer were present in retinas showing the greates amount of damage (panels e-h). No pyknotic nuclei were observed in these retinas which accounted for 25% or fewer of the cases in each group. Least damaged retinas showed some variability in appearance (panels a-d); however, these differences were within the range of those observed across the control retinas. Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer; GCL - ganglion cell layer. Calibration bar = 150 µm.

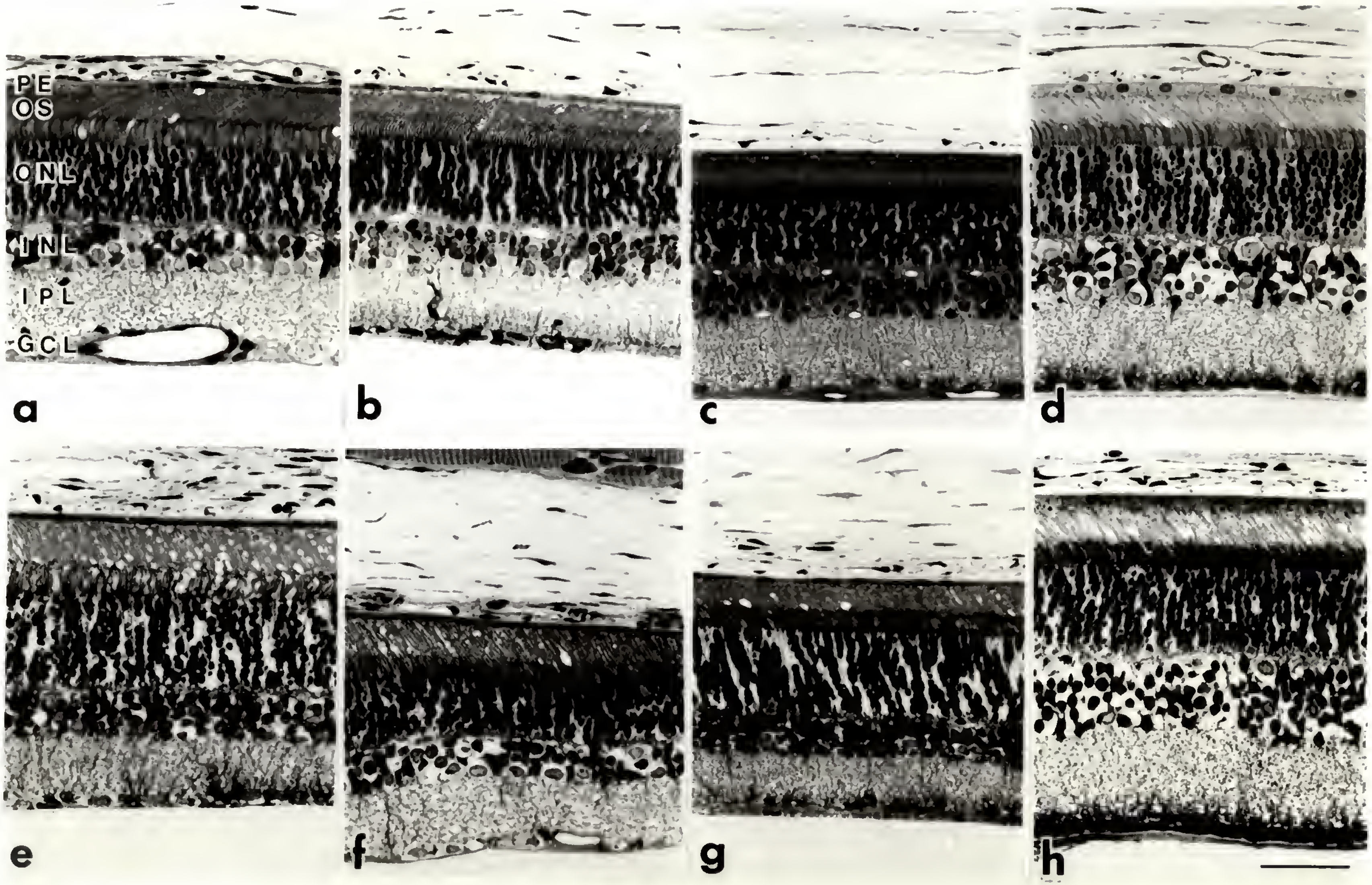
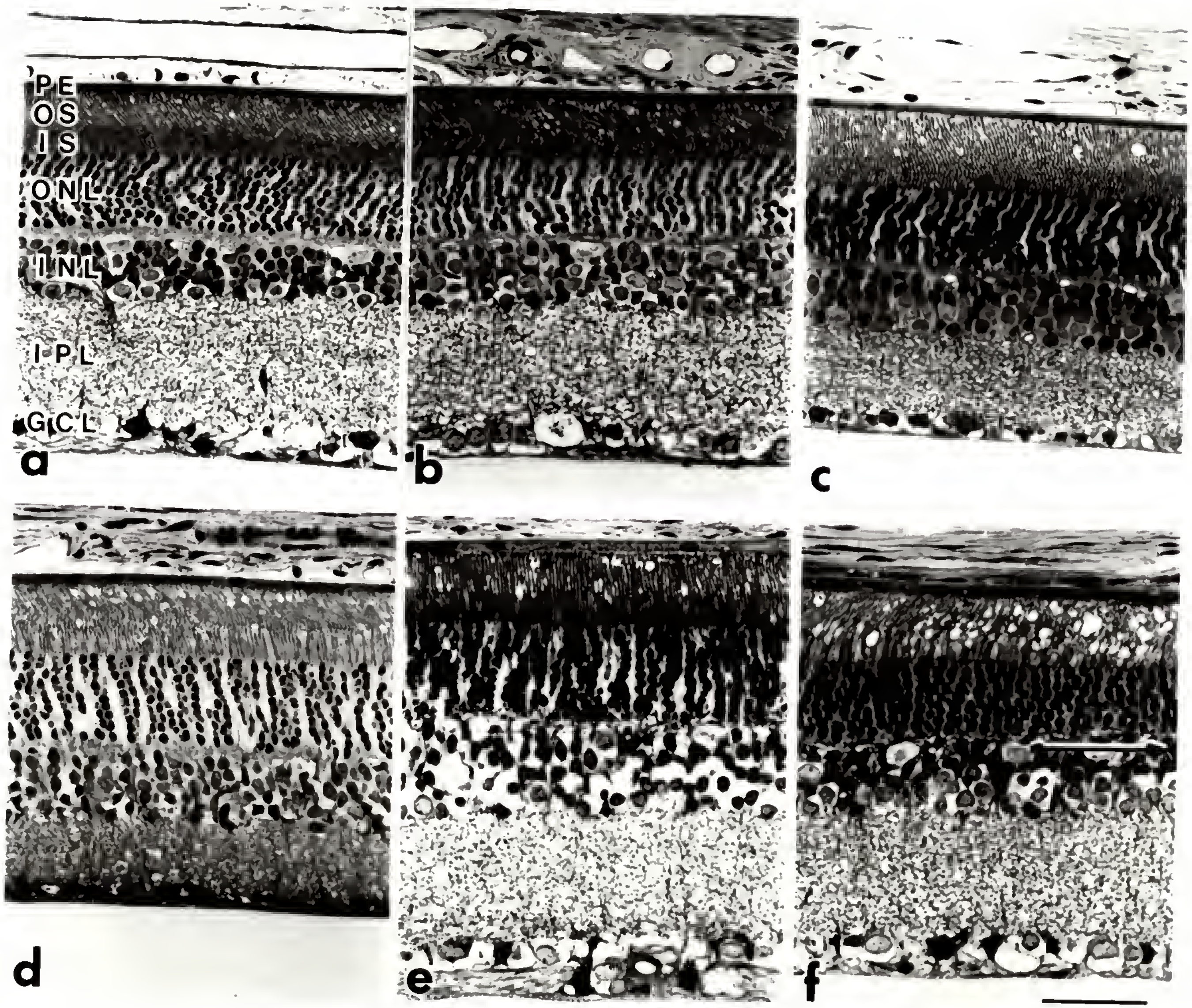

PE
OS
ONL
INL
IPL
GCL
a
b
c
d
e
f
g
h

Figure 3-15. Light photomicrographs of retinal tissue taken from animals exposed to 65 lux. Panels a-c were taken of retinas of animals exposed to 65 lux for 28, 59 or 85 days, respectively, and are representative of retinas showing the least amount of damage under these light exposure conditions. Panels d-f are representative of retinas showing the greatest amount of damage under these exposure conditions. All photographs were taken of the SON region. The majority of the retinas showed little evidence of light damage (66% or greater of cases examined in each group) (panels a-c). Vesiculated membranes in OS layer (panels d-f) and swollen inner segments (IS layer in panel f) were evident in severely damaged retinas. Loss of photoreceptor nuclei from the outer nuclear layer (region above arrow, panel f) was evident following 85 days exposure in cases of severest damage. Abbreviations: PE - pigment epithelium; OS - photoreceptor outer segments; IS - photoreceptor inner segments; ONL - outer nuclear layer; INL - inner nuclear layer; IPL - inner plexiform layer; GCL - ganglion cell layer. Calibration bar = 150 µm.


PE
OS
IS
ONL
INL
IPL
GCL
a
b
c
d
e
f

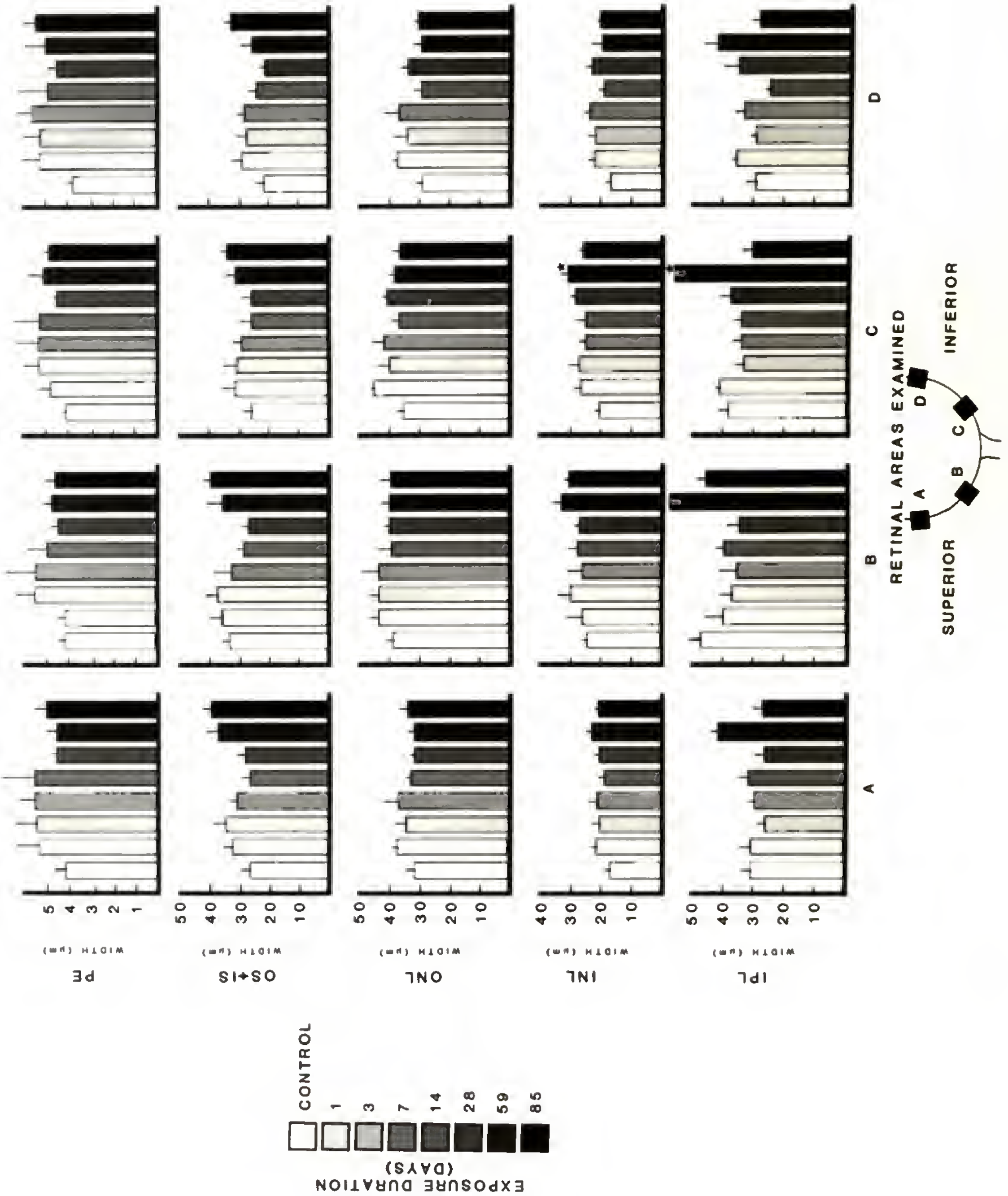
and e). The most severely damaged retinas in each of these groups accounted for 33% or fewer of the cases examined. The least damaged retinas in these groups showed no evidence of light damage at the light microscopic level.

Finally, the SON region of retinas taken from animals exposed to 65 lux for 85 days are shown in Figure 3-15, c and f. Examination of histology taken from a severely damaged retina (Figure 3-15f) revealed loss of photoreceptor nuclei from the outer nuclear layer in a region underlying that which contained highly vesiculated photoreceptor outer segments. The corresponding histology taken from a least damaged retina (Figure 3-15d) showed little evidence of light damage and was representative of 66% of the cases examined (4 out of 6 cases).

Morphometric analyses of the retinas of animals exposed to 65 lux for 1, 3, 7, 14, 28, 59 or 85 days were carried out to determine the effect of experimental light exposure on the widths of the various layers of the retina. The mean widths obtained for the five retinal layers measured in each of the four regions of the retinas examined are shown in Figure 3-16.

Mean values calculated for the control and experimental groups were compared within each of the four retinal regions using 20 separate one way analyses of variance followed by Bonferroni t-tests with the significance level set at $p=0.05$. In general, the results of these analyses showed that there were no differences between the widths of the

Figure 3-16. Results of morphometric analyses of retinas of animals exposed to 65 lux for 1, 3, 7, 14, 28, 59 or 85 days (12:12 L:D). The control group was maintained under 6 lux (12:12 L:D). The widths of five retinal layers (um) were determined in each of four retinal regions (A = SUP, B = SON, C = ION and D = INF). Each bar represents the group mean (+ or - SE). Experimental group means marked with an asterisk (*) were significantly different ($p=0.05$) from the corresponding control group means. Abbreviations: PE - pigment epithelium; ONL - outer nuclear layer; OS+IS - photoreceptor outer plus inner segments; INL - inner nuclear layer; IPL - inner plexiform layer.


PE
OS+IS
ONL
INL
IPL
WIDTH (µm)
5
4
3
2
1
50
40
30
20
10
A
B
C
D
RETINAL AREAS EXAMINED
SUPERIOR
INFERIOR
EXPOSURE DURATION (DAYS)
CONTROL
1
3
7
14
28
59
85
*

control and experimental retinal layers in the four regions examined.

The results of the between group comparisons of the widths of the five retinal layers showed that there was a significant between groups effect for the inner plexiform layer in all four retinal regions examined. A significant between groups effect was also obtained for the retinal layer containing the photoreceptor inner and outer segments in the SUP retinal region and for the inner nuclear layer in the ION retinal region (see Table 3-3 for ANOVA results). Post-hoc tests on mean inner plexiform layer widths determined for the ION region of the retina revealed that the value calculated for animals exposed to 65 lux for 59 days was significantly greater than the corresponding mean control value. The mean inner plexiform layer widths determined for animals exposed to 65 lux for 1, 3, 7, 14, 28 or 85 days were not different from the control value in this region. Post-hoc tests on mean inner plexiform layer widths determined for the SUP, SON and INF regions of the retina revealed that the values calculated for the groups of experimental animals were not different from the corresponding control values. Post-hoc tests on mean inner nuclear layer widths determined for the ION region of the retina revealed that the value calculated for animals exposed to 65 lux for 59 days was significantly greater than the corresponding mean control value. The mean inner nuclear layer widths determined for the other experimental groups in this region were not different from the inner nuclear layer width determined for the control group. Finally, post-hoc tests on the mean widths of the retinal layer containing the photoreceptor inner and outer segments determined for the SUP region of the retina revealed that the values calculated for the animals exposed to 65 lux were not different from the control value.

The results obtained from semi-quantitative comparisons of the retinas of animals exposed to 65 lux for 1, 3, 7, 14, 28, 59 or 85 days with the retinas of control animals revealed that differences could be observed in the SUP regions of the retinas of the animals exposed to 65 lux. Little difference was observed between experimental and control animals in the SON, ION and INF regions of the retina. Mean total histological scores obtained for each of the groups in the four retinal regions examined are shown in Table 3-4. These scores were compared to the corresponding control scores using t-tests with the significance level set at $p=0.05$.

The results of these comparisons, which are shown in Table 3-5, revealed that mean scores for the SUP regions of retinas of animals exposed to 65 lux for 3, 28, 59 or 85 days were significantly greater than the corresponding mean control score. The mean score obtained for the INF region of the retinas of animals exposed to 65 lux for 59 days was significantly greater than the corresponding control score. All mean scores obtained for the SON and ION regions of the experimental retinas were not different from the corresponding mean control scores.

3.3 Discussion

The effects of 65, 130, 270 and 1345 lux on the physiology and morphology of the albino rat retina were examined in the present study in order to determine the minimum light intensity which produces retinal damage. Retinas were considered to be damaged if either the physiological or the morphological evaluations of the retinas of the experimental animals were found to be significantly different from the control group evaluations following three or more consecutive exposure periods. Based on the results of these experiments, it appears that a reasonable estimate for the threshold light intensity which produces damage to the retinas of albino rats raised under 6 lux (12:12 L:D) lies somewhere between 65 and 130 lux, or approximately one log unit above the light intensity under which the animals were raised. These light levels are similar to those found under street lights at night.

The analyses of the data obtained from the animals exposed to either 65 or 130 lux suggested that transient changes in the morphology and/or responsiveness of the retinas obtained from both groups did occur during the course of the 85 day test period. Electrophysiological data obtained from the animals exposed to these two light intensities showed some significant variation from the control data over time, but these differences were not observed for more than two of the consecutive exposure periods examined. Similar results were obtained from the morphometric and semi-quantitative analyses of the retinal tissue histology obtained from these two groups of animals; however, fewer significant differences were observed between the control animals and those exposed to 65 lux. Concomitant changes were observed in the morphology and electrophysiology of the retinas of animals exposed to 130 lux for 3 days. These retinas showed significant increases in both the latencies of the b-wave components of the ERGs and in the widths of some of the retinal layers relative to the corresponding control values. These particular changes may have been causally related; however, caution must be exercised in proposing causality since the remaining morphological and physiological changes in this group were not paired with respect to time. In general, the evidence for light damage to retinas of animals exposed to 130 lux (12:12 L:D) was stronger than that obtained for animals exposed to 65 lux. For these reasons, a value between 65 and 130 lux was chosen as the estimate for the minimum light intensity which produces retinal damage.

The results of the analyses of the data obtained from animals exposed to 270 lux clearly show that this light intensity produces damage to the retinas of albino rats raised under 6 lux. It would appear, based on the data obtained for the amplitudes of the b-wave components of the ERGs recorded from these animals, that 3-7 days exposure to this light intensity is sufficient to cause significant changes in the functioning of the retina which are maintained throughout the remaining portion of the 59 day test period.

The latencies of the b-waves recorded from the animals exposed to 270 lux were also significantly different from those obtained from the control group after 3-7 days exposure; however, these changes were not consistently observed during the remaining portion of the test period. Following 14 or 28 days exposure to 270 lux the latencies of the b-waves were not different from the control values, but 59 days exposure resulted in a significant increase in the experimental latency values. One possible explanation for the return of the latency values to control levels following 14 or 28 days exposure to 270 lux is that most of the damaged photoreceptor cells have been removed from the retinas of these animals by these times. Removal of debris would increase the rate of ion movement through the retina and would result in a decrease in the latency of the responses of the cells of the retina. The histological results obtained from these groups of animals lend support to this hypothesis (see Figure 3-9, d and e which represent 50-80% of the cases examined).

Semi-quantitative evaluations of the histology of the retinal tissue obtained from animals exposed to 270 lux support the conclusion that 3-7 days exposure to this light intensity is sufficient to cause damage. The morphometric data suggest a more conservative estimate of the time needed to produce retinal damage. These data suggest that a minimum of 28 days exposure to 270 lux is needed before significant changes are observed in the morphology of the experimental animals' retinas. The morphometric analyses of the retinas exposed to 270 lux showed that the damage was limited to the layers of the retina containing the photoreceptor cells and outer segments and that there was a reduction in the width of the outer nuclear layer due to cell loss which began after 3-7 days exposure to 270 lux and became significant after 28 days exposure. Donnelly and O'Steen (1982) have shown that quantitative changes which occur in the morphology of the retina following toxic light exposure are not fully manifest until approximately 14 days after exposure. These authors attribute the observed delay to the time lag between cell damage and the removal of the damaged cells from the retina. This delay may be an important factor in explaining the discrepancy between the electrophysiological and the morphological estimates for the length of exposure to 270 lux needed to produce retinal damage. Since photoreceptor cell loss appears to begin following 3-7 days exposure to 270 lux and the electrophysiological and semi-quantitative estimates for the length of exposure needed to produce damage range from 3-7 days, it seems reasonable to conclude that the retinas of albino rats raised under 6 lux are damaged with 3-7 days of exposure to 270 lux, a light level which approximates that present at the surface of a well illuminated writing desk.

Finally, the analyses of the electrophysiological and anatomical data obtained from animals exposed to 1345 lux suggest that this light intensity severely damages the retinas of albino rats raised under 6 lux in 7 days or less. Damage appeared to be limited to the layers of the retina containing the photoreceptor cells. In addition, damage was most severe in the SON region of the retina. This result confirms the previous report of Rapp and Williams (1980) who showed that the superior portion of the albino rat retina is most sensitive to the effects of light damage.

The 65-130 lux estimate for the minimum cyclic light intensity which produces damage to the retinas of albino rats raised under 6 lux obtained in the present study is quite similar to the 60 lux value reported by Stötzer et al. (1970). The present results differ from those of O'Steen et al. (1972) and Carter-Dawson et al. (1982) who have reported that 6 month exposures to cyclic light intensities of 194 lux and 107 lux, respectively, do not damage the albino rat. One or several of the factors which have been shown to influence the extent of the structural changes associated with retinal light damage may underlie the differences in the reported affects of various light intensities on the rat retina.

In the present study, all animals were raised from birth under carefully controlled lighting conditions. This practice is exercised by only a few investigators in this field. Penn and Williams (1986) recently reported that the light history of albino rats influences rod outer segment length, photoreceptor cell density, whole retina rhodopsin concentrations and the regeneration rate of rhodopsin. They also reported that the photon catching rates of retinas of albino rats raised under cyclic illuminances differing by more than two orders of magnitude were statistically equal. Based on their results, Penn and Williams (1985) suggest that the photoreceptors of the albino rat retina adjust the composition of the outer segment membranes in order to achieve an optimal level of function. If, during development, photoreceptor cells adjust to perform optimally under the existing ambient light levels and these adjustments are maintained throughout the cells' lifetime, then the light history of an animal may be an extremely important factor in determining the susceptibility of the retinas of albino rats to light damage.

One may speculate that differences in the light histories of the various experimental animals used in studies of light damage may be primarily responsible for the variability observed among these studies. Perhaps if information was provided to investigators concerning the light conditions under which the animals they purchased were reared and if this information was included in research reports, some of the variability in the light damage literature could be eliminated or considered in interpretations of the results. The ideal situation would be one in which investigators raise their experimental animals from birth so that lighting conditions throughout the animals life could be controlled by the investigator.

CHAPTER 4
EFFECTS OF DIM CYCLIC LIGHT ON THE PIGMENT EPITHELIUM

4.1 Introduction

The degenerative changes which occur in the retina in response to toxic light exposure have been described by many investigators. Recently, Noell (1980) has described two different kinds of retinal pathology caused by light in the albino rat.

The first kind of degeneration is characterized histologically by widespread photoreceptor layer abnormalities and loss of visual cells. The pigment epithelium remains intact and its cells have been reported to be comparable in appearance to those in control animals both at the light (Rapp and Williams, 1980) and electron microscopic level (O'Steen and Lytle, 1971; Shear et al., 1973). Damage to the photoreceptor cell layer is not uniform throughout the retina and appears to be most pronounced in an area superior to the optic nerve head (Rapp and Williams, 1980). This type of damage is observed under a variety of conditions but is most prevalent in studies where the body temperature of the rat remains normal and low intensity lights are used (O'Steen and Lytle, 1971; Rapp and Williams, 1980; Shear et al., 1973).

The second kind of degeneration is characterized histologically by degenerative changes in both the photoreceptor layer and the pigment epithelium. The degenerative changes in the pigment epithelium occur prior to or simultaneously with those in the photoreceptor layer (Grignolo et al., 1969; Hansson, (1970); Kuwabara, 1970; Noell et al., 1966). As in the first kind of degeneration, the damage to the pigment epithelium and photoreceptors is most severe in the central regions of the retina with less severe changes in peripheral regions (Grignolo et al., 1969; Noell, 1980; Noell et al., 1966; O'Steen and Karcioglu, 1974). The vulnerability of retinal tissues to this kind of damage is greatly enhanced by exposing albino rats to long durations of high intensity light during periods of elevated body temperature (Grignolo et al., 1969; Kuwabara, 1970; Noell et al., 1966; O'Steen and Karcioglu, 1974). Other factors which influence the extent of damage are age (Kuwabara and Funahashi, 1976; O'Steen et al., 1974), and previous light history (Noell, 1979, 1980).

The morphological changes that occur in each of these two types of light-induced retinal degeneration are similar. In both, degenerative changes of the photoreceptor cells begin with vesiculation of the outer segment membranes and end with the death and removal of the cells from the retina by cells of the pigment epithelium, macrophages of vascular origin and glial cells (O'Steen and Karcioglu, 1974; O'Steen and Lytle, 1971).

The major difference between these types of degeneration is that cells of the pigment epithelium do not die in the first type of degeneration. Ultrastructural studies do reveal that the pigment epithelium in the first type of damage contains increased numbers of cytoplasmic phagosomes and smooth endoplasmic reticulum, and that the villous processes are disoriented and retracted during the various stages of photoreceptor degeneration (Kuwabara, 1970; Shear et al., 1973). In the second type of degeneration, the cells of the pigment epithelium progress through a number of histolopathological changes which culminate in the deaths of the cells. Ultrastructural signs of light damage to these cells include edema, vacuolation and rupture of subcellular organelles (Hansson, 1970; Kuwabara, 1970).

Studies which suggest that the pigment epithelium is not permanently altered by low intensity light base their conclusions on the results of morphological analyses of this tissue. These studies do not preclude the possibility that low intensity light could cause physiological changes in the pigment epithelium which would not be detected using morphological techniques.

The physiological status of the pigment epithelium may be assessed by recording both ERG c-waves and sodium azide-induced changes in the resting potential of the eye. The c-wave is believed to originate across the pigment epithelium (Niemeyer, 1973; Noell, 1953, 1954; Steinberg et al., 1970) and results from hyperpolarization of these cells in response to a light-evoked decrease in the concentration of extracellular potassium in the subretinal space (Oakley and Green, 1976; Oakley, 1977; Steinberg et al., 1980). Changes in the amplitude or latency of the c-wave of the ERG would, therefore, indicate changes in the functioning of the pigment epithelium. The second functional test used an intravenous injection of sodium azide. This agent causes a transient rise in the resting potential of the eye (Noell, 1952, 1953, 1963; Pautler and Noell, 1976; Weidner, 1976) which Noell (1952, 1953, 1963) has proposed to be due to its affects on active transport processes across the basal membranes of the pigment epithelium cells. This potential, which apparently arises from interference with ion exchange processes across the membranes of the cells of the pigment epithelium, provides a second measure of the integrity of the pigment epithelium which does not require receptor participation or a light stimulus.

The present study examines the effects of low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination) on the function of the pigment epithelium of albino rats. An intensity of 6 lux (12:12 L:D) was chosen because albino rats raised under higher light intensities (160 lux) have been reported to lack d.c. ERG c-waves (Graves et al., 1985) and the results could be compared to data obtained from the other studies in this disseration. The results obtained from albino rats raised from birth under 6 lux are compared to those obtained from two control groups: albino rats raised under minimal light exposure conditions (dark-reared;

exposed to 0.1 lux during three 5 minute periods per week) and pigmented rats raised in a commercial facility, then housed in our facility under 6 lux cyclic light. Pigmented rats were chosen as a second control group because of their known relative insensitivity to light damage (Rapp and Williams, 1980).

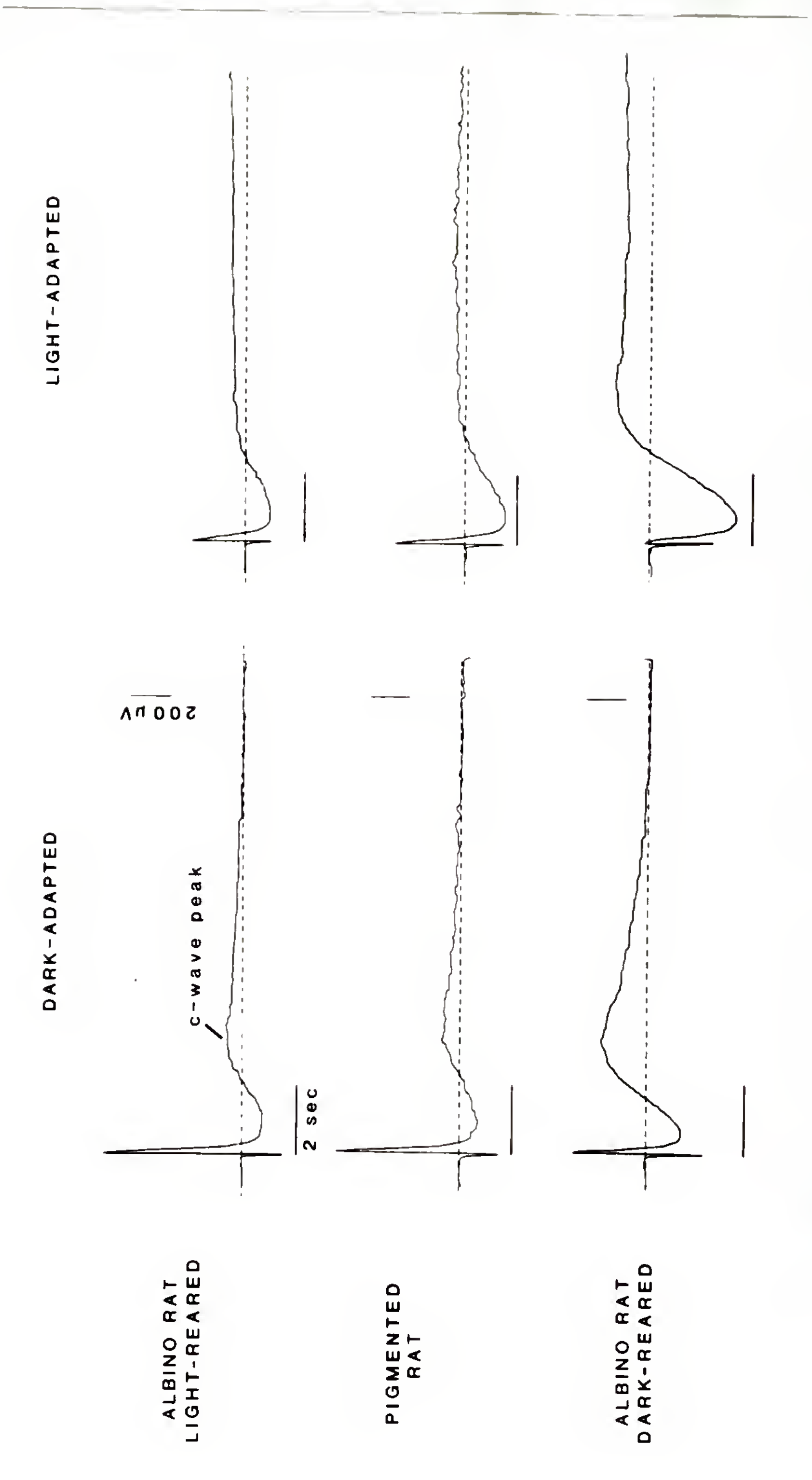
4.2 Results

4.2.1 Electrophysiological Results

4.2.1.1 The ERG c-wave. Representative ERGs recorded from an albino rat raised under low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination), a dark-reared albino rat and a pigmented rat are shown in Figure 4-1. All traces shown were obtained in response to a 25 μsec xenon lamp flash (intensity = -0.08 log fL·sec). All ERGs recorded following 20 minutes dark adaptation possessed a c-wave, the largest of which was recorded from the dark-reared albino rat. The ERGs recorded from the albino rat raised under low intensity cyclic light and from the pigmented rat were quite similar. The ERGs recorded following 20 minutes light adaptation to 0.1 lux also possessed measurable c-waves; however, the amplitudes of these responses were reduced compared to those recorded following dark adaptation. As was the case after dark adaptation, the largest c-wave was recorded from the dark-reared albino rat.

The mean amplitudes and latencies of the c-wave component of the ERGs recorded from the three experimental

Figure 4-1. Typical d.c. ERGs recorded from an adult pigmented rat and adult albino rats raised under either minimal light exposure conditions (dark-reared) or low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination). Responses on the left side of the figure were recorded following a 20 minute adaptation period to complete darkness. Responses on the right side of the figure were recorded following a 20 minute adaptation period to 0.1 lux. The stimulus used to elicit these responses was a 25 µsec flash of white light (intensity = -0.08 log fL·sec).


DARK-ADAPTED
LIGHT-ADAPTED
c-wave peak
200 µV
2 sec
ALBINO RAT LIGHT-REARED
PIGMENTED RAT
ALBINO RAT DARK-REARED

groups were compared at each of the three flash intensities and two adaptation levels tested using six separate one way analyses of variance, followed by Duncan's multiple range tests with the significance levels set at p=0.05. The data on the amplitudes of the c-waves that were recorded from the three experimental groups in this study are summarized in Figure 4-2 and showed that there was a significant between groups effect in all cases except in the comparison of the responses of the groups to the lowest intensity flash (-1.48 log fL•sec) following dark adaptation (see Table 4-1 for ANOVA results). Post-hoc tests on the means revealed that the c-waves recorded from dark-reared albino rats were significantly larger than those recorded from either pigmented rats or albino rats raised under low intensity cyclic light following either dark adapation or light adaptation. Responses recorded from pigmented rats were not different from those recorded from albino rats raised under low intensity cyclic light.

Mean latencies of the c-waves recorded from the three experimental groups are shown in Figure 4-2. The between group comparisons of the latencies of the c-waves elicited to the two lowest flash intensities following dark adaptation were found to be significant (see Table 4-2 for ANOVA results). In both cases, post-hoc tests on the means revealed that the c-wave latency values obtained for dark-reared albino rats were significantly greater than those obtained for albino rats raised under low intensity

Figure 4-2. Mean [+ or - SE] amplitudes [A] and latencies [B] of ERG c-waves recorded from pigmented rats and albino rats raised under either minimal light exposure conditions [dark-reared] or low intensity cyclic light [12:12 L:D; 6 lux ambient cage illumination]. Responses were measured to three flash intensities following both a 20 minute adaptation period to complete darkness and a 20 minute adaptation period to 0.1 lux. A] * Mean c-wave amplitude for dark-reared albino rats was found to be significantly greater [p=0.05] than values obtained from either pigmented rats or albino rats raised under low intensity cyclic light. B] * Mean c-wave latency for dark-reared albino rats was found to be significantly greater [p=0.05] than the corresponding value obtained fro albino rats raised under low intensity cyclic light. Abbreviations: ALR - albino light reared; ADR - albino dark reared; PIGMT - pigmented.

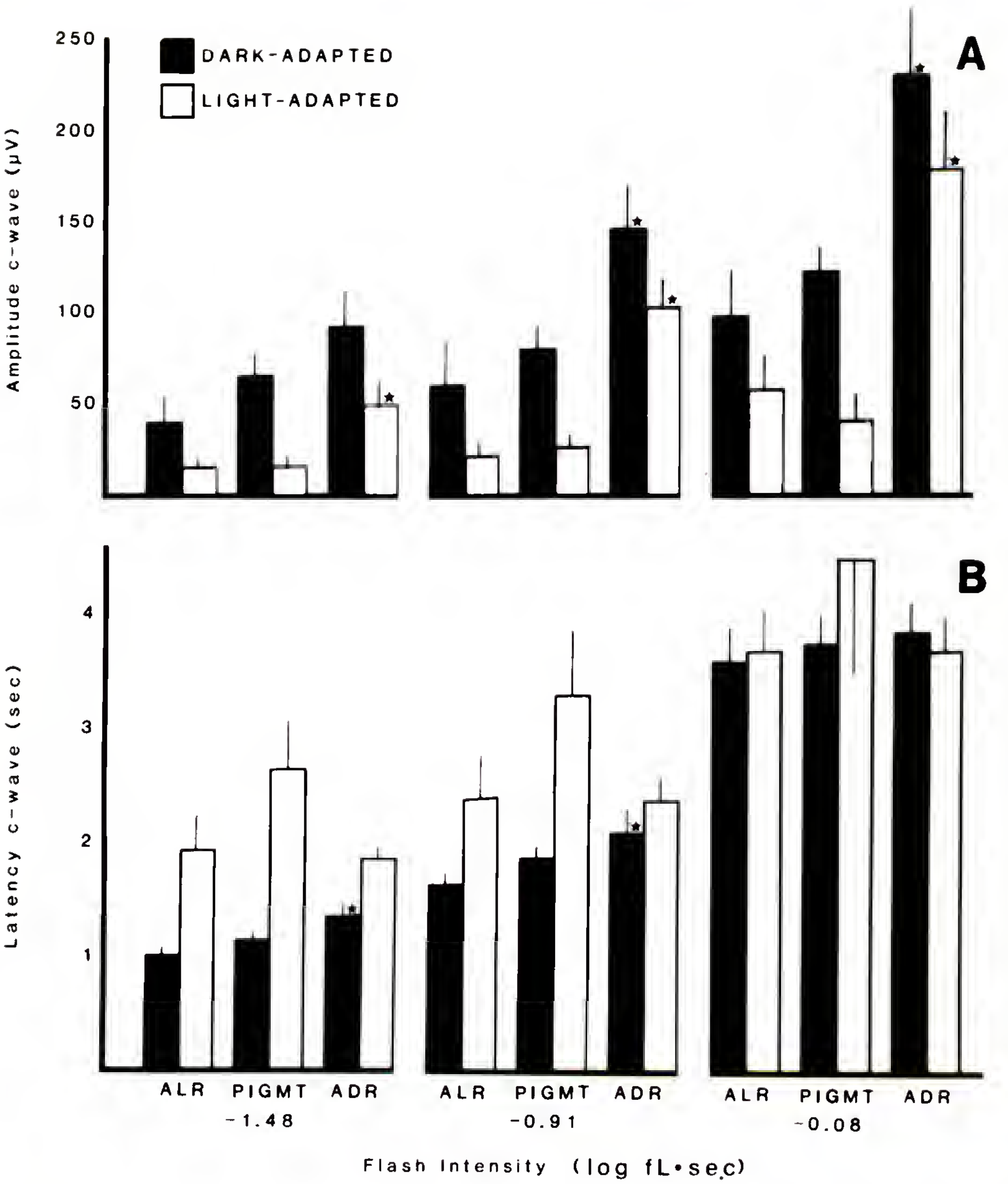

A
B
DARK-ADAPTED
LIGHT-ADAPTED
Amplitude c-wave (µV)
250
200
150
100
50
Latency c-wave (sec)
4
3
2
1
ALR
PIGMT
ADR
-1.48
-0.91
-0.08
Flash Intensity (log fL•sec)

TABLE 4-1

COMPARISONS OF MEAN ERG C-WAVE AMPLITUDES RECORDED FROM PIGMENTED RATS, DARK-REARED ALBINO RATS AND LIGHT-REARED ALBINO RATS WITHIN ADAPTATION STATE AND FLASH INTENSITY USING ONE WAY ANALYSES OF VARIANCE [ANOVA]

ADAPTATION STATE		FLASH INTENSITY [log fL·sec]		
		-1.48	-0.91	-0.08
	[df]	[2,29]	[2,28]	[2,29]
DARK-ADAPTED		2.93†	4.72 *	6.26 **
	[df]	[2,28]	[2,26]	[2,28]
LIGHT-ADAPTED		5.41 **	14.93 ***	12.30 ***

†ANOVA F value
* p<.05
** p<.01
*** p<.001

cyclic light. The latencies of the c-waves recorded from the pigmented rats were not different from those recorded from either albino group.

The decrease in the amplitudes of the c-waves recorded from albino rats raised under low intensity cyclic light relative to those recorded from dark-reared albino rats could be a result of light damage to either the neural retina or pigment epithelium or both. In order to test the hypothesis that these low levels of cyclic light caused damage to the neural retina, a comparison was made of the amplitudes and latencies of the b-waves of the ERGs recorded from the three experimental groups.

4.2.1.2 The ERG b-wave. The mean amplitudes and latencies of the b-wave component of the ERGs recorded from the three experimental groups were compared at each of the three flash intensities and two adaptation levels tested using six separate one way analyses of variance, followed by Duncan's multiple range tests with the significance levels set at $p=0.05$. Mean amplitudes of the ERG b-waves recorded from the three experimental groups are shown in Figure 4-3. The analyses of these data showed that the mean amplitudes of the b-waves recorded following dark adaptation were not different between groups. The amplitudes of the b-waves recorded following light adaptation did show a significant between groups effect (see Table 4-3 for ANOVA results). Post-hoc tests on the means revealed that the amplitudes of the b-waves recorded from dark-reared albino

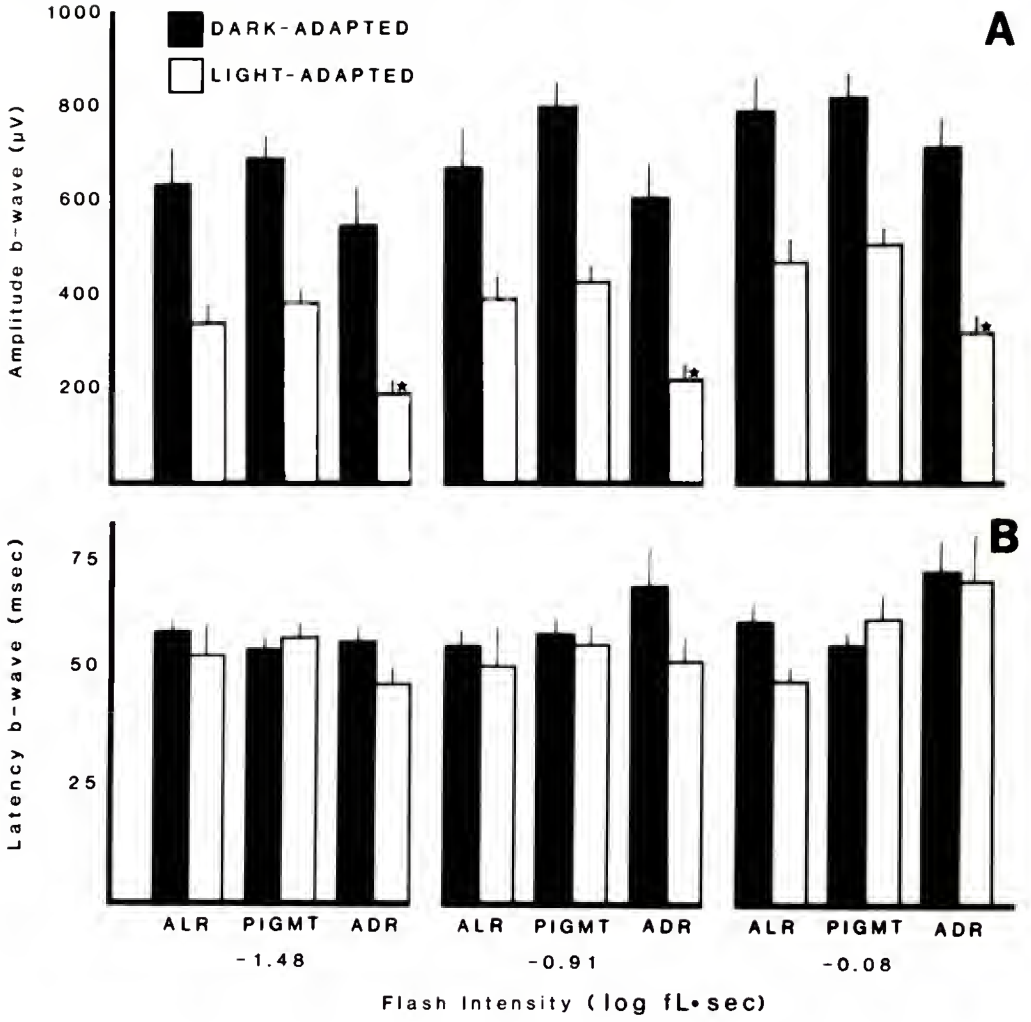
TABLE 4-2

COMPARISONS OF THE MEAN LATENCIES OF THE C-WAVES RECORDED FROM PIGMENTED RATS, DARK-REARED ALBINO RATS AND LIGHT-REARED ALBINO RATS WITHIN ADAPTATION STATE AND FLASH INTENSITY USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

ADAPTATION STATE		FLASH INTENSITY (log fL·sec)		
		-1.48	-0.91	-0.08
	(df)	(2,26)	(2,26)	(2,29)
DARK-ADAPTED		4.08†*	3.88 *	.22
	(df)	(2,18)	(2,19)	(2,22)
LIGHT-ADAPTED		2.66	2.03	.75

†ANOVA F value
* p<.05[

Figure 4-3. Mean (+ SE) amplitudes (A) and latencies (B) of ERG b-waves recorded from pigmented rats and albino rats raised under either minimal light exposure conditions (dark-reared) or low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination). Responses were measured to three flash intensities following both a 20 minute adaptation period to complete darkness and a 20 minute adaptation period to 0.1 lux. A) * Mean b-wave amplitude for dark-reared albino rats was found to be significantly smaller (p=0.05) than values obtained from either pigmented rats or albino rats raised under low intensity cyclic light following light adaptation. B) Mean b-wave latencies for the three experimental groups were not found to be different from each other. Abbreviations: ALR - albino light reared; ADR - albino dark reared; PIGMT - pigmented.


A
B
DARK-ADAPTED
LIGHT-ADAPTED
Amplitude b-wave (µV)
1000
800
600
400
200
Latency b-wave (msec)
75
50
25
ALR
PIGMT
ADR
-1.48
-0.91
-0.08
Flash Intensity (log fL•sec)
*

rats were significantly smaller than those recorded from either pigmented rats or albino rats raised under low intensity cyclic light.

The mean b-wave latency data are shown in Figure 4-3. The mean latencies of the b-waves recorded from the three experimental groups were not different from each other (see Table 4-4 for ANOVA results).

4.2.1.3 Sodium azide-induced changes in the resting potential of the eye. In order to test the hypothesis that low intensities of cyclic light caused damage to the pigment epithelium, we recorded changes in the resting potential of the eye in response to a low dose bolus of intravenous sodium azide. Representative azide responses recorded from the three experimental groups are shown in Figure 4-4. Azide responses were obtained from all experimental animals tested and were characterized by a rapid, transient, positive rise in the resting potential. The mean amplitudes of the azide responses obtained from each of the experimental groups are shown in Figure 4-5. Mean azide responses were compared using one way analysis of variance followed by a Duncan's multiple range test with the significance level set at p=0.05. Azide responses did show a significant between groups effect [F(2,29) = 4.83, p=0.0154] and the post-hoc test on the means revealed that the responses recorded from albino rats raised under low intensity cyclic light were significantly smaller than those recorded from pigmented rats or dark-reared albino rats.

TABLE 4-3

COMPARISONS OF MEAN ERG B-WAVE AMPLITUDES RECORDED FROM PIGMENTED RATS, DARK-REARED ALBINO RATS AND LIGHT-REARED ALBIND RATS WITHIN ADAPTATION STATE AND FLASH INTENSITY USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

ADAPTATION STATE		FLASH INTENSITY (log fL·sec)		
		-1.48	-0.91	-0.08
	(df)	(2,29)	(2,29)	(2,29)
DARK-ADAPTED		1.13†	1.49	.68
	(df)	(2,29)	(2,28)	(2,28)
LIGHT-ADAPTED		10.51 **	9.92 **	5.66 *

†ANOVA F value

* p<.01

** p<.001

TABLE 4-4

COMPARISONS OF THE MEAN LATENCIES OF THE B-WAVES RECORDED FROM PIGMENTED RATS, DARK-REARED ALBINO RATS AND LIGHT-REARED ALBINO RATS WITHIN ADAPTATION STATE AND FLASH INTENSITY USING ONE WAY ANALYSES OF VARIANCE (ANOVA)

ADAPTATION STATE		FLASH INTENSITY (log fL·sec)		
		-1.48	-0.91	-0.08
	(df)	(2,29)	(2,29)	(2,29)
DARK-ADAPTED		.39[†]	.80	2.31
	(df)	(2,29)	(2,28)	(2,28)
LIGHT-ADAPTED		1.60	.13	2.66

[†] ANOVA F value

* $p<.05$

Figure 4-4. Representative transient, positive potential changes recorded from a pigmented rat and albino rats raised under minimal light exposure conditions (dark-reared) or low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination) following intravenous injection of sodium azide. The potential change started approximately 0.5-1.0 sec after initiating the bolus azide injection.

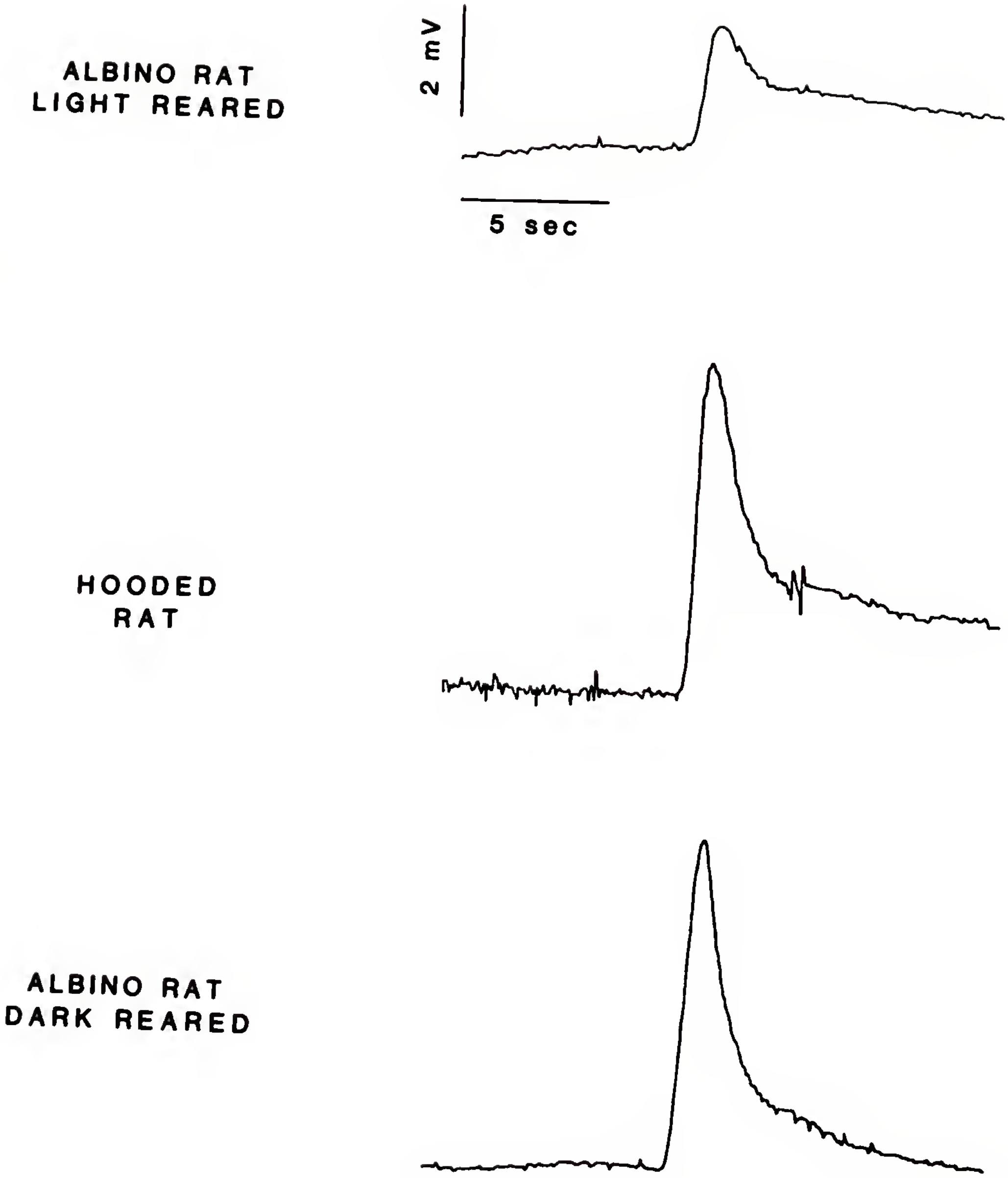
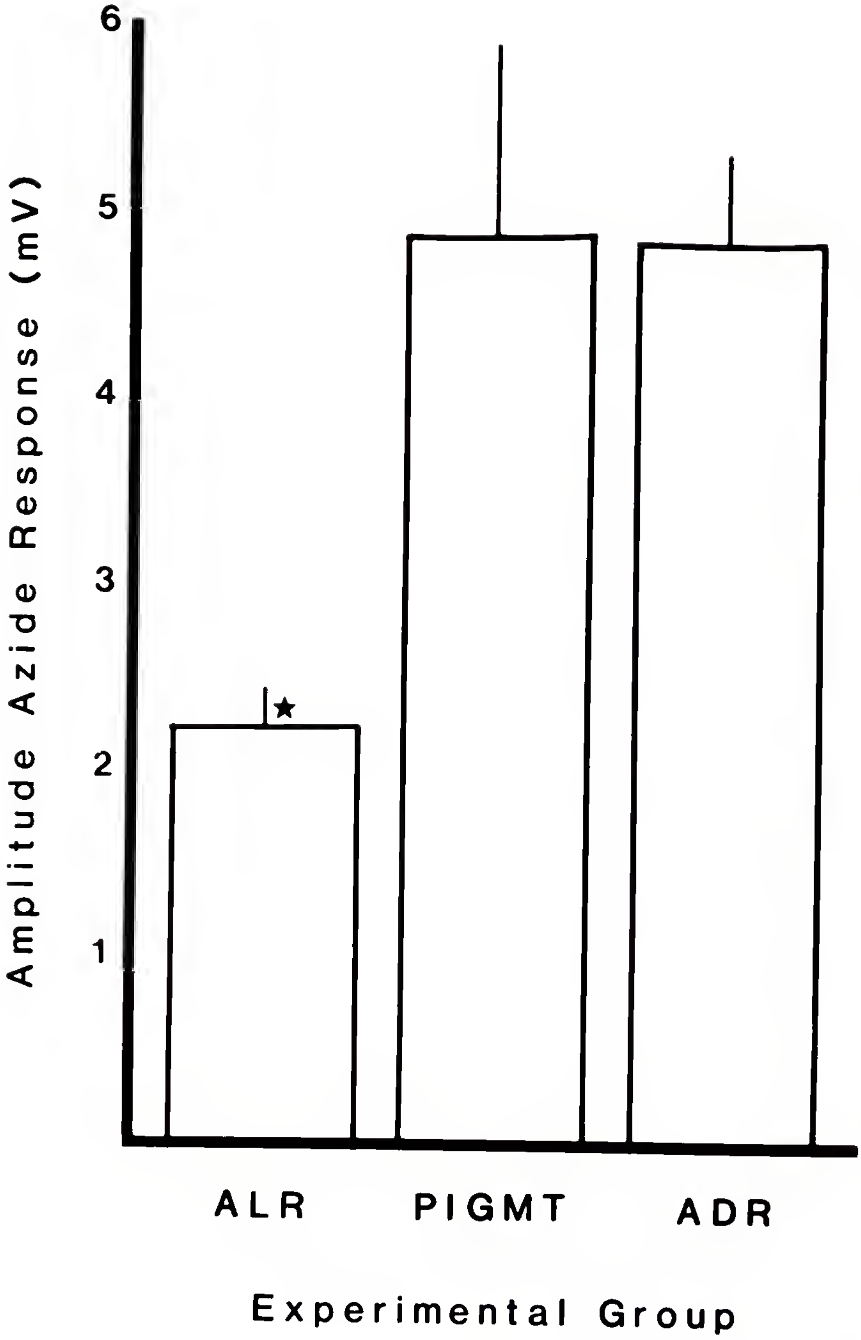

ALBINO RAT
LIGHT REARED
2 mV
5 sec
HOODED
RAT
ALBINO RAT
DARK REARED

Figure 4-5. Mean (+SE) amplitudes of resting potential changes caused by sodium azide injection. * Mean azide response for albino rats raised under low intensity cyclic light (n=11) was found to be significantly smaller (p=0.05) than values obtained from either pigmented rats (n=10) or dark-reared albino rats (n=10). Abbreviations: ALR - albino light reared; ADR - albino dark reared; PIGMT - pigmented.


Amplitude Azide Response (mV)
6
5
4
3
2
1
★
ALR
PIGMT
ADR
Experimental Group

The responses recorded from pigmented rats were not different from those recorded from dark-reared albino rats.

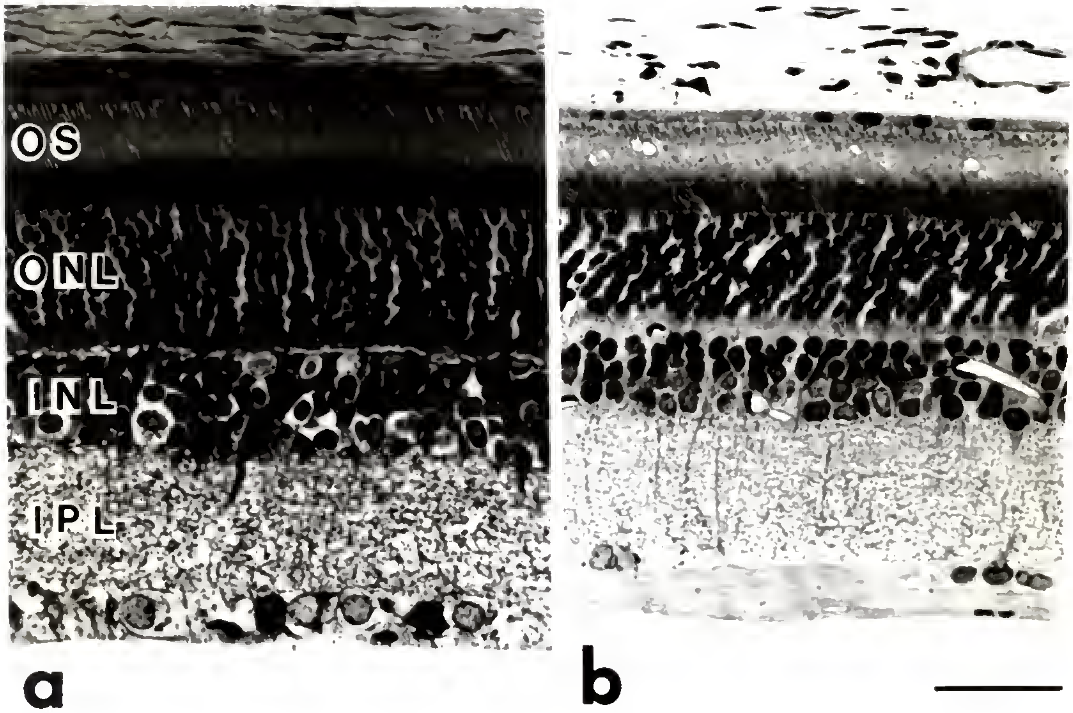
4.2.2 Histological Results

Retinas taken from albino rats raised under low intensity cyclic light were examined using light microscopic techniques to determine whether morphological changes associated with light damage were present. These retinas were qualitatively compared to those obtained from dark-reared albino rats. Attention was focused on the retinal region extending from 700-2000 µm superior to the optic nerve head in the vertical meridian. Examples of retinal histology obtained from this region for dark-reared albino rats and albino rats raised under low intensity cyclic light are shown in Figure 4-6. Both the retina taken from the albino rat raised under low intensity cyclic light (Figure 4-6b) and the retina taken from the dark-reared albino rat (Figure 4-6a) possess 8-10 rows of photoreceptor nuclei in their outer nuclear layers, and the photoreceptor outer segments and the pigment epithelia appear normal in both cases. No observable histopathological changes in either the photoreceptors or the pigment epithelia of albino rats raised under low intensity cyclic light were found.

4.3 Discussion

The present study showing that dark-reared albino rats possess prominent c-waves confirms the findings reported by other investigators (Graves et al., 1985; Pautler and Noell, 1976). In addition, it shows for the first time that albino

Figure 4-6. Light photomicrographs of retinal tissue taken from the retinas of albino rats raised under (a) minimal light exposure conditions or (b) under low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination). The photographs were taken of the SON retinal region located 1500 μm superior to the optic nerve head. Both retinas possess 8-10 photoreceptor nuclei in the outer nuclear layer, and in each, the appearance of the photoreceptor outer and inner segments is unremarkable. Calibration bar = 150 μm.


OS
ONL
INL
IPL
a
b

rats possess c-waves that are comparable in amplitude and latency to those of pigmented rats if the albino rats are raised under low intensity cyclic light (12:12 L:D; 6 lux ambient cage illumination). The responses recorded from light-reared albino rats were found to be consistently smaller than those recorded from dark-reared albino rats. Other studies have shown that albino rats raised under the higher light levels (160-430 lux) lack d.c. ERG c-waves (Graves et al., 1985). It therefore appears that the intensity of light under which albino rats are raised is an important factor in determining the magnitude of the ERGs recorded from these animals.

Several mechanisms could explain the reductions observed in the amplitudes of the c-waves recorded from albino rats raised under cyclic light. The c-wave of the ERG is due to a light-evoked hyperpolarization of the apical membrane of the pigment epithelium (Steinberg et al., 1970; Steinberg and Miller, 1973) which is believed to occur in response to a decrease in the extracellular concentration of K+ around the photoreceptor (Oakley and Green, 1976). Light may, therefore, damage the photoreceptors and cause an accumulation of outer segment debris in the subretinal space which could form a barrier to ions moving between the photoreceptors and the pigment epithelium. Alternatively, the ability of the photoreceptors to cause a decrease in extracellular potassium levels may be compromised as a result of light exposure. Finally, light may alter the cells of the pigment epithelium and therefore affect K^+ conductance across them.

The first possibility seems unlikely since the photoreceptors of animals raised under 6 lux cyclic illumination did not show histological evidence of light damage at the light microscopic level. There may have been changes in these cells not visible at the light microscopic level, but no accumulation of outer segment debris in the subretinal space or vesiculation of the membranous discs comprising the photoreceptor outer segments was observed in these retinas.

Physiologically, one of the first manifestations of light damage to the retina is a decrease in the amplitude and an increase in the peak-latency of the ERG b-wave for a given light stimulus [Kuwabara and Gorn, 1968; Noell et al., 1966]. The b-wave is generated by the Müller cells in response to changes in the ionic composition of the extracellular fluid [Miller and Dowling, 1970] and provides an indirect measure of photoreceptor activity. If the function of the photoreceptors was compromised by light exposure, one would expect to see changes in the b-waves of animals raised under 6 lux illumination. No differences were found in the amplitudes or latencies of b-waves recorded from rats raised under 6 lux compared to those recorded from dark-reared albino rats following 20 minutes of dark adaptation. There was, however, a significant reduction in the amplitudes of the b-waves recorded from the dark-reared albino rats following 20 minutes of light adaptation. Depression of the amplitude of the ERG b-wave has been observed in several species in experiments designed to investigate the effects of visual deprivation [Cornwell and Sharpless, 1968; Ganz et al., 1968; Hamasaki and Flynn, 1973; Hamasaki and Pollack, 1972]. Hypotheses concerning the mechanism underlying this effect have been proposed but they remain speculative. Since a decrease in the amplitudes or an increase in the peak latencies of the ERG b-waves recorded from the albino rats raised under 6 lux was not observed, one may conclude that the photoreceptors in these retinas have not been functionally compromised by exposure to light.

In order to test the third hypothesis, the effects of sodium azide on the resting potential across the eye were measured. The results of this experiment showed that the azide responses recorded from albino rats raised under low intensity cyclic light were significantly smaller than those recorded from either dark-reared albino rats or pigmented rats housed under cyclic light. Noell [1952, 1953, 1963] has proposed that the transient rise in the resting potential of the eye in response to sodium azide is due to the effects of azide on active transport processess across the basal membrane of the pigment epithelium. If the mechanism proposed by Noell is correct, then the data obtained in this experiment indicate that low levels of cyclic light impair transport processes across the abino rat pigment epithelium.

In contrast, light microscopic examination of the retinas of albino rats raised under 6 lux did not suggest that the pigment epithelium had been structurally altered by light. Care must be exercised in interpreting these results since many of the earliest morphological manifestations of light damage can only be detected using ultrastructural techniques (Grignolo et al., 1969; Hansson, 1970; O'Steen and Karcioglu, 1974; Shear et al., 1973). If low intensity cyclic light affects ion channels or transport processes in the cells of the pigment epithelium as the results of the azide experiment suggest, then morphological analyses of these tissues using ultratructural techniques may fail to indicate a change. In this case, the electrophysiological index may be the most sensitive marker available.

Ultrastructural and biochemical studies conducted by Hansson (1970, 1971) and more recently by Lin and Essner (1985) provide evidence supporting the hypothesis that cells of the albino rat pigment epithelium are altered by light. Hansson (1971) measured the activity of various oxidative enzymes in albino rat retina following light damage and found that the cells of the pigment epithelium showed a damage and found that the cells of the pigment epithelium showed a reduction in the activity of cytochrome oxidase, diaphorases and dehydrogenases within the first few hours of exposure. Similar changes were observed somewhat later in the inner segments of the photoreceptors. Ultrastructural changes observed to occur during the first few hours of light exposure included swelling and loss of basal membranous processes from the cells of the pigment epithelium (Hansson, 1970). The light intensities used by Hansson in his investigations were not given; however, the rapid loss of photoreceptor cells implies that relatively high intensities of light were used. Lin and Essner (1985) studied the effects of low intensity cyclic light (11 lux, 12:12 L:D) on the ultrastructure of the basal membrane of the pigment epithelium in albino rats and found that the infoldings of the basal membrane were flattened over approximately 6% of longitudinal retinal sections. Occasional focal exaggerations of the basal and lateral infoldings of the pigment epithelial cells were also observed. The data obtained in the present experiment, which suggest that transport processes across the pigment epithelium are impaired by low levels of cyclic light, may represent the functional manifestation of the ultrastructural changes observed by Hansson (1970) and by Lin and Essner (1985) in the basal membrane of the albino rat pigment epithelium.

The pigment epithelium has so far received little attention in studies investigating the pathology and etiology of light-induced retinal damage. Most of the studies conducted in this area have focused on the retinal layers containing the photoreceptor cells. The electrophysiological results obtained in this experiment suggest that the pigment epithelium of albino rats is altered by extremely low levels of light. Perhaps these changes in the pigment epithelium precede those observed in the neural retina in response to toxic light exposure.

CHAPTER 5
EFFECTS OF TOXIC LIGHT ON PHOTORECEPTOR RENEWAL

5.1 Introduction

The principal change which occurs in the retina as a result of exposure to toxic light is the degeneration and eventual death of the photoreceptor cells [Noell et al., 1966; O'Steen and Lytle, 1971; Shear et al, 1973]. The majority of light damage studies have shown that the primary site of damage to the photoreceptor cell is the outer segment which is the light sensitive region of these cells [Kuwabara, 1970; Noell et al., 1966; O'Steen and Lytle, 1971; Shear et al., 1973]. The outer segment is comprised of a number of membranous discs which are stacked in an orderly array within the limiting outer segment plasma membrane. Following exposure to toxic light, the outer segment disc membranes become disrupted, showing separation and formation of tubules and vesicles. If light exposure is continued, the damaged outer segments are eventually phagocytized and photoreceptor cell death often ensues [Henton and Sykes, 1983; O'Steen and Karcioglu, 1974; O'Steen and Lytle, 1971; Shear et al., 1973].

The structural integrity of the adult photoreceptor outer segment is normally maintained through the complementary processes of membrane disc synthesis and degradation. New membranes are formed by evagination of the ciliary plasma membrane at the base of the outer segment (Steinberg et al., 1980). These membranes are closed and then apically displaced as additional membranous discs are formed. To complete the process, the discs reaching the apical tip of the outer segment are removed and degraded by the overlying pigment epithelium (Droz, 1963; Young, 1967; Young and Droz, 1968).

The processes underlying the turnover of outer segment disc membrane have been under investigation since the discovery that mature photoreceptor outer segments constantly replenish their protein and lipid components (Droz, 1963; Young, 1967; Young and Droz, 1968). Autoradiography of retinal tissue following an injection of radioactive amino acids has shown that nearly all new protein molecules are synthesized in association with ribosomes in the myoid region of the inner segment. (Young and Droz, 1968). The major protein produced in rods is opsin, the transmembrane glycoprotein moiety of the rod visual pigment (Bargoot et al., 1969; Hall et al., 1968; Hargrave, 1982). The polypeptide component is synthesized on rough endoplasmic reticulum (Hall et al., 1969; Young and Droz, 1968), cotranslationally inserted into the rough endoplasmic reticulum membrane (Goldman and Blobel, 1981) and then routed through the Golgi apparatus (Papermaster et al., 1978; Young and Droz, 1968) where carbohydrate groups are added prior to transport to the ciliary region.

The mechanism of delivery and accumulation of opsin in the photoreceptor outer segment is still not understood. Recent morphological, freeze-fracture, immunocytochemical and autoradiographic data (Besharse and Forestner, 1981; Besharse and Pfenninger, 1980; Defoe and Besharse, 1985; Papermaster et al., 1979, 1985) have identified a population of smooth membranous vesicles and cisternae in the pericilliary region which have been proposed to be the principal organelles of opsin transport to the site of disc assembly. The events in the ciliary region which lead to disc morphogenesis are less well defined. Evidence obtained from freeze-fracture studies suggests that the membranous vesicles containing newly synthesized opsin fuse with the plasma membrane in the pericillary region prior to being transported to the outer segment via the ciliary plasma membrane (Andrews, 1982; Besharse and Pfenninger, 1980).

Normally, the photoreceptor outer segments of rats and mice are entirely renewed within approximately 9-10 days (LaVail et al., 1972; Young, 1967). In frogs (Young, 1967), dogs (Buyukmihci and Aguirre, 1976) and monkeys (Young, 1971) the renewal process occurs in 42, 6 and 9-13 days, respectively.

Several studies have investigated the effects of various light regimes on photoreceptor renewal in amphibian and mammalian retinas (Basinger et al., 1976; Battelle and LaVail, 1978; Besharse, 1980; Besharse and Hollyfield, 1979; Besharse et al., 1977a, 1977b; Currie et al., 1978;

Hollyfield et al., 1980; Hollyfield et al., 1982; Goldman et al., 1980; Young, 1967). In amphibians, light has been found to accelerate disc synthesis to a variable extent depending on species, ambient temperature and state of dark-adaptation (Besharse et al., 1977_a_, 1977_b_; Young, 1967). Under constant light conditions, shedding rates were found to be reduced while maximum rates of disc synthesis were maintained, the result of which was a rapid and dramatic increase in the lengths of the photoreceptor outer segments (Besharse et al., 1977_b_). Constant darkness also affects the renewal of amphibian photoreceptor outer segments. Both disc synthesis and shedding rates decreased under conditions of constant darkness, the result of which was an increase in the lengths of the rod outer segments because the decreases in the two processes were unequal (Basinger et al., 1976; Bridges et al., 1976).

In constrast to results obtained in amphibians, observations concerning rod outer segment turnover in pigmented rodents suggest a greater degree of intrinsic regulation of disc synthesis and shedding independent of environmental lighting conditions. For example, Besharse and Hollyfield (1979) found that constant light or constant dark conditions had little effect on disc synthesis or shedding rates in the pigmented mouse retina. In studies on the effects of dark adaptation on rhodopsin content and rod outer segment length in pigmented rat eyes, Battelle and LaVail (1978) found that the rhodopsin content did not increase appreciably after 10 days in constant darkness. On the other hand, the results of several research reports suggest that the renewal process in the albino rat retina may be influenced by changes in environmental lighting (Baker et al., 1986; Battelle and LaVail, 1978; Goldman et al., 1980; Young, 1967).

Young (1967) reported that the rate of disc synthesis in the albino rat retina was slightly elevated under conditions of constant illumination. Constant or increased intensities of light have also been reported to reduce shedding and phagocytosis rates in the albino rat retina (Goldman et al., 1980; Baker et al., 1986). In studies on the effects of 10 days constant darkness on the rhodopsin content of the eyes of albino rats, Battelle and LaVail (1978) found that the rhodopsin content of the eyes of animals kept in the dark increased by approximately 50% and the lengths of the rod outer segments increased by as much as 32% over values obtained from animals maintained in cyclic light. The nature of the imbalance in the renewal process which resulted in these increases is not yet understood.

The renewal process consists of a complicated series of events which, if adversely affected, could seriously interfere with the normal functioning of the photoreceptor cell. Results of studies investigating the effects of photoperiod on photoreceptor renewal in the albino rat retina suggest that the balance between the processes of disc synthesis and degradation may be disrupted by changes in environmental lighting. The aim of the present study was to determine if rod outer segment renewal rates in albino rat retinas are affected by increases in environmental illumination which produce retinal damage. Evaluation of photoreceptor renewal in retinas during the early stages of light damage is important since an imbalance in this process could contribute to the degeneration of the light damaged photoreceptor cell.

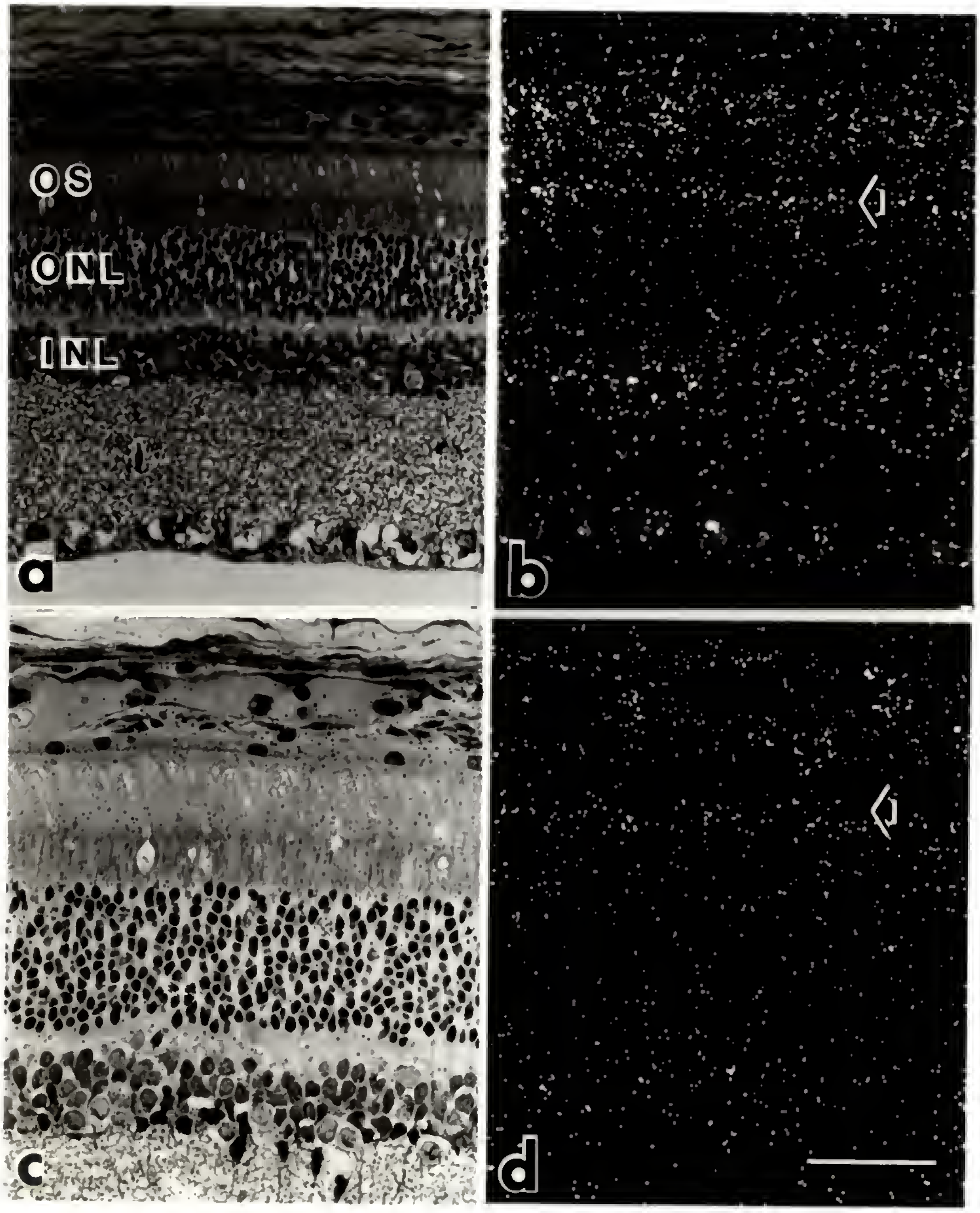
Autoradiographic techniques were employed in the present study to examine membrane renewal rates in two experimental groups and one control group over a seven day test period. Experimental animals were exposed to either 130 lux or 270 lux (12:12 L:D) and control animals were maintained under 6 lux (12:12 L:D). Membrane renewal rates were determined from measurements of radioactive band (^{3}H-band) displacement along the lengths of the outer segments in the SON region of each retina as a function of time.

5.2 Results

Examples of the autoradiograms obtained in this experiment are shown in Figure 5-1. The photomicrographs were taken from the SON region of the retina of an experimental animal exposed to 130 lux for 3 days (12:12 L:D) and sacrificed 60 hours after an injection of radio-labelled amino acids. Figure 5-1, b and d are dark-field photomicrographs of the same field as shown in Figure 5-1, a and c, respectively. Examination of the layer of the retina containing the photoreceptor outer segments in the SON regions of experimental and control animals revealed the presence of an area with a relatively high density of silver grains whose leading edge moved toward the apical tips of the photoreceptor outer segments with time (Figure 5-1, b and d, arrows). The pattern of band displacement was similar in experimental and control groups. Twelve hours after injection of labelled amino acids, the leading edge of the silver grain band was positioned over the base of the photoreceptor outer segments. Sixty hours after injection, the leading edge of the silver grain band had been apically displaced approximately 10 µm from the base of the outer segment. Finally, 156 hours after injection , the leading edge of the silver grain band had been apically displaced approximately 25 µm from the base of the outer segment. In some cases, the ION region was also examined to see if there may have been regional differences in the responses of the photoreceptor cells. Based on these observations, there was no difference in the pattern of band displacement across the retina.

Linear regression analyses were carried out to determine the rate of renewal of the rod outer segments in the experimental and control animals over the 7 day period tested. These data are plotted in Figure 5-2. The regression equations obtained for the three groups were as follows: (1) control group, distance = $-0.9 + 0.2t$, (2)

Figure 5-1. Light photomicrographs of autoradiograms. All photomicrographs were taken of the retina of an animal exposed to 130 lux for 3 days (12:12 L:D) and sacrificed 60 hours after an injection of radiolabelled amino acids. All photographs were taken of the SON region located 1500 µm superior to the optic nerve head. Panels b and d are dark-field photomicrographs of the regions shown in panels a and c, respectively. An area of relatively high grain density can be seen over the layer of the retina containing the photoreceptor outer segments (arrows, panels b and d). Abbreviations: OS - photoreceptor outer segments; ONL - outer nuclear layer; INL - inner nuclear layer. Calibration bar = 122 µm for panels a and b, and 150 µm for panels c and d.


OS
ONL
INL
a
b
c
d

experimental animals exposed to 130 lux, distance = 1.02 + 0.13t and [3] experimental animals exposed to 270 lux, distance = -0.64 + 0.15t where t is time in hours. Renewal values calculated for a 24 hour period from these equations were [1] control group, 3.9 μm, (2) animals exposed to 130 lux, 4.14 μm and (3) animals exposed to 270 lux, 2.96 μm. Two way analysis of variance of the data obtained from the autoradiograms for band dispacement distances as a function of time (Figure 5-2) revealed that there was no significant difference between data from the experimental and control groups [F(2,22) = 2.56, p>0.1]. As expected, the main effect at time was highly significant [F(2,22) = 91.53, p<0.0001], but the group x time interaction was not significant [F(4,22) = 2.14, p>0.1].

Silver grain densities were determined over the photoreceptor outer segment layers in the SON regions of the experimental and control retinas as a function of time post-injection (Figure 5-3). Two way analysis of variance of the mean densities for the experimental and control groups calculated as a function of time revealed that there was no significant difference between the experimental and control group values [F(2,22) = 0.03]. Similarly, the main effect at time [F(2,22) = 2.42, p>0.1] and the group x time interaction [F(4,22) = 3.05, p=0.053] were not significant.

5.3 Discussion

The effects of 6, 130 and 270 lux cyclic light on outer segment renewal rates in retinas of rat raised under 6 lux

Figure 5-2. Migration distances of silver grains over photoreceptor outer segments, during exposure to 130 or 270 lux, as a function of hours survived following injection of labelled amino acids. Each point represents the mean migration distance (+SE). Best fit lines to the data were determined using linear regression. The following equations were calculated: control, y= -0.9 + 0.2t; animals exposed to 130 lux, y= 1.02 +0.13t; animals exposed to 270 lux, y= -0.64 + 0.15t where y = distance in um and t = time in hours. The mean outer segment length (+SE) for all groups was 34.4 + 2 μm.

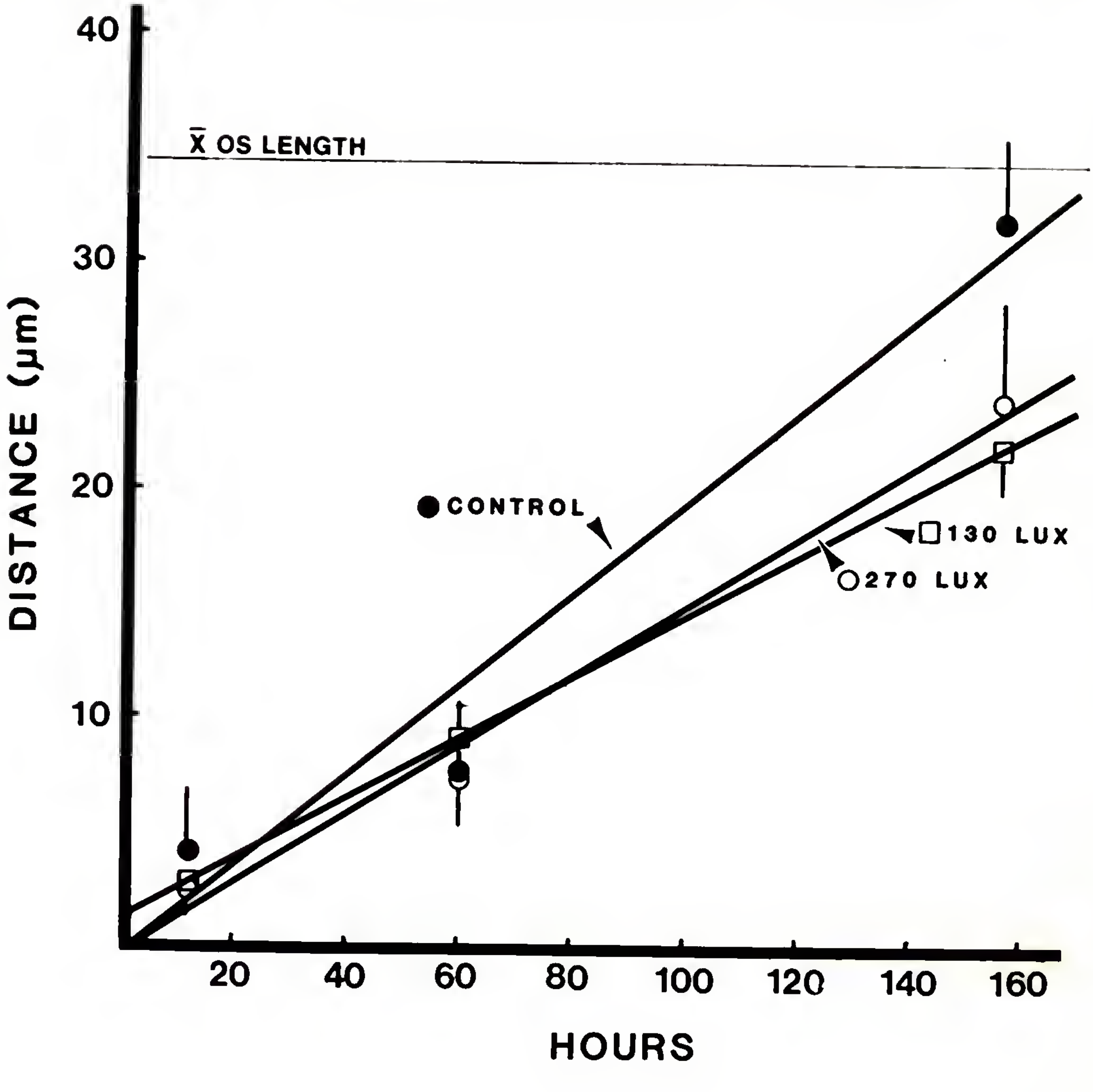
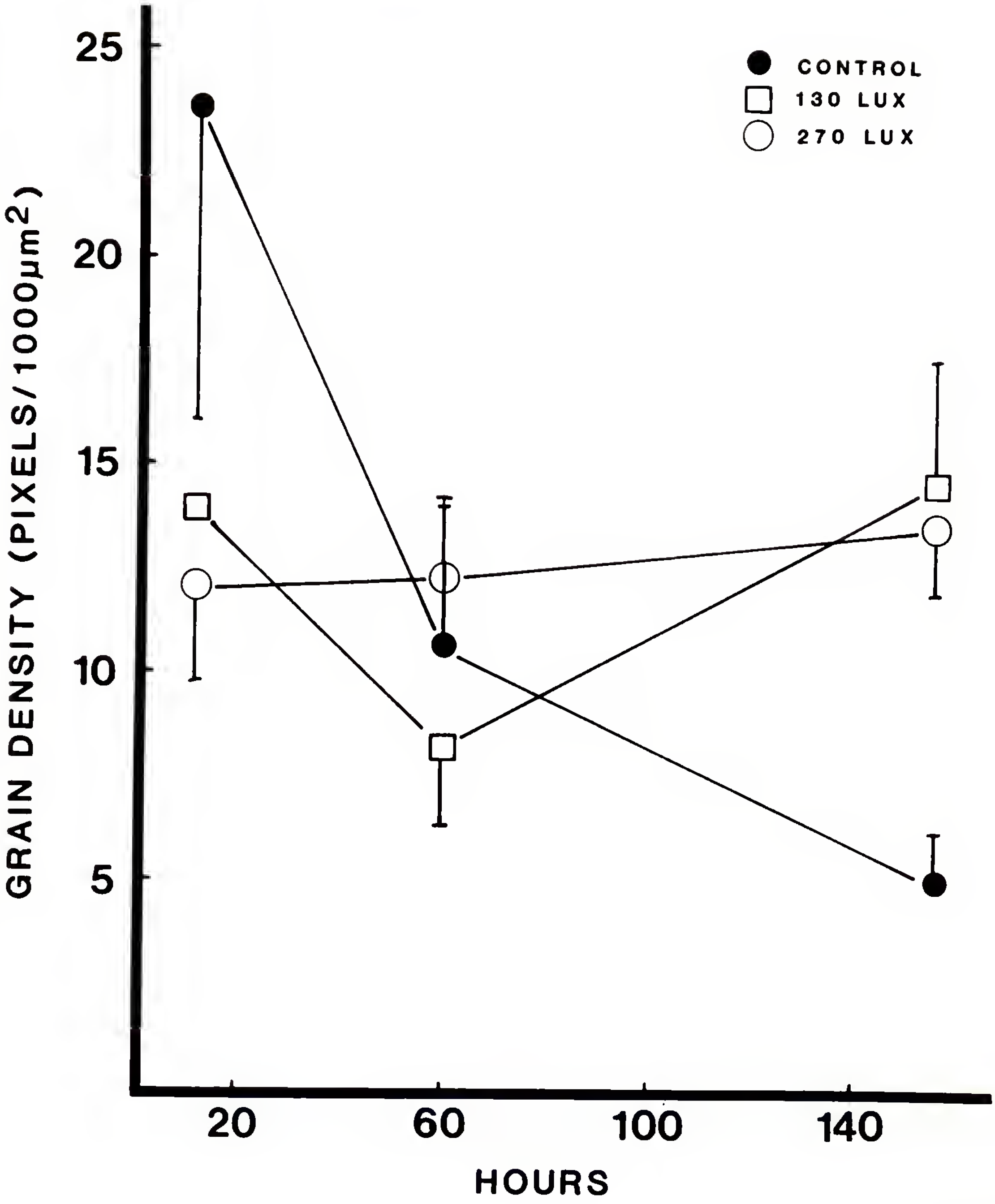

X̄ OS LENGTH
● CONTROL
□ 130 LUX
○ 270 LUX
DISTANCE (μm)
10
20
30
40
HOURS
20
40
60
80
100
120
140
160

Figure 5-3. Silver grain densities over the photoreceptor outer segments in the retinal region located 1500 μm superior to the optic nerve head following exposure to 130 or 270 lux. All points represent group means [+ or - SE]. Two way analysis of variance of the mean densities for the experimental and control groups calculated as a function of time revealed that there were no significant differences observed between the experimental and control group values.


● CONTROL
□ 130 LUX
○ 270 LUX
GRAIN DENSITY (PIXELS/1000µm²)
25
20
15
10
5
20
60
100
140
HOURS

were determined in this study. The results of experiments presented in CHAPTER 3 of this dissertation showed that one week exposure to light intensities of 130 and 270 lux (12:12 L:D) produce mild to moderate levels of retinal damage in albino rats raised under 6 lux.

Renewal of the rat rod outer segment has been found by previous investigators to take approximately 9-10 days (LaVail et al., 1972; Young, 1967). Based on data obtained in the present study, renewal of the rod photoreceptors in the control and the experimental animals exposed to 130 or 270 lux was estimated to take approximately 8.8, 8.3 or 11.6 days, respectively. Renewal rates were estimated from measurements taken in the SON regions of the retinas. The SON region of the albino rat retina was chosen for the analysis since it is this region which has been demonstrated to be most susceptible to light damage (Rapp and Williams, 1980). Analyses of the renewal data showed that the rates of renewal for the three groups were not statistically different from each other. It appears, therefore, that the rate of renewal of the rod outer segment does not change in retinas exposed to levels of environmental light which produce retinal damage.

The results of the present study are similar to those reported by Wyse (1980), who determined the rate of synthesis of new outer segment membrane in the retinas of albino rats which had been damaged by exposure to 1020 lux light for 24 hours by measuring the amount of new disc membrane added to the damaged outer segments as a function of time in constant darkness. Retinal sections stained with toluidine blue were examined at the light microscopic level and identification of new and damaged membranes was based on the differences in the staining characteristics of each. The animals in his study had been raised from birth under 215 lux (14:10 L:D). Wyse (1980) determined that the renewal of the damaged photoreceptor outer segments in albino rat retinas recovering from light damage was approximately 8.8 days. The present study together with Wyse's suggest that photoreceptor cells do not change their rates of outer segment renewal in response to or during recovery from exposures to toxic light.

Young (1967) reported that the rate of outer segment disc synthesis in the albino rat was slightly elevated under conditions of constant illumination. The findings of the present study do not support Young's results. Young (1967) used 15 days of constant light at an intensity of 6500 lux to investigate the effects of light on the renewal process. The experimental light intensities used in the present study (130 and 270 lux) were chosen because they produce mild to moderate levels of light damage in the retina without causing significant loss of photoreceptor cells. Experiments described in CHAPTER 3 revealed that as few as 8 days exposure to 1345 lux (12:12 L:D) caused a significant reduction in the number of photoreceptor cells in the SON region of the retina and clearly damaged the outer segment layer throughout the retina. Based on these data, exposure to intensities of 1345 lux or higher would render measurements of renewal rates difficult if not impossible since the method used depends on the presence of orderly outer segments. The lighting regime used by Young now seems extreme in view of what is currently known concerning the effects of light intensities on the albino rat retina, and therefore, care should be taken in comparing his results to those obtained in the present study.

Estimates of synthesis of membrane protein by the photoreceptor cells were made by determining silver grain densities over the photoreceptor outer segments in the SON regions of the experimental and control retinas. The variability in these measurements was observed to be, in some cases, quite large. Factors which may have contributed to this variability are (1) small sample size (n=2-4), (2) differences in tissue preparation and (3) differences in the amount of labelled amino acids available to the retinas of the animals. Statistical analysis of these data showed that the silver grain densities for the three groups were not different from each other. The results of this analysis suggest that increased light intensities do not significantly affect the amount of protein incorporated into photoreceptor cell outer segments.

In conclusion, the rate of photoreceptor renewal does not appear to be affected by light intensities shown to cause retinal damage. The results of this study do not address the question of whether light intensities which damage the retina alter the relative amounts of lipid and protein synthesized by photoreceptor cells. Further studies of the synthesis of the various membrane components of the outer segment membrane may be necessary before the affect of toxic light on photoreceptor renewal processes can be fully understood.

CHAPTER 6
CONCLUSIONS

This study investigated the effects of various intensities of cyclic light on the retina of the albino rat. The objectives were (1) to define those light conditions which would produce damage to the retinas of albino rats raised under 6 lux (12:12 L:D), (2) to determine what effects low intensity cyclic light has on the functioning of the albino rat pigment epithelium and (3) to determine whether the rate of photoreceptor outer segment renewal is affected by light conditions which produce either mild or moderate levels of damage to the albino rat retina.

Previous studies have suggested that the threshold for light damage to the albino rat retina is low relative to that found for other animals (Kuwabara and Gorn, 1968; Lanum, 1978; Rapp and Williams, 1980). The present study demonstrates that the retinas of albino rats raised under 6 lux (12:12 L:D) are easily damaged by light intensities not much higher than the light intensity under which the animals are raised (CHAPTER 3). A 1.0-1.3 log unit increase in the cyclic light intensity produced transient changes in the morphology and responsiveness of the retina over a period of 85 days, while an increase of 1.6 log units produced dramatic changes in the morphology and responsiveness of the retina within 3-7 days (CHAPTER 3).

Recently, the Committee on Care and Use of Laboratory Animals (1985) published a revision of their Guide for the Care and Use of Laboratory Animals which includes new recommendations for animal facility lighting levels. In the past, illumination levels for animal rooms of 807-1076 lux have been recommended, light levels which have been shown to cause light damage to retinas of albino mice (Greenman et al., 1982) and rats (Stötzer et al., 1970 and the present study). The Committee on Care and Use of Laboratory Animals now recommmends that the level of illumination approximately 1 meter above the floor in rooms housing animals be approximately 323 lux. The results of this study (CHAPTER 3) suggest that this light level, which approximates that present at the surface of a well illuminated writing desk, will cause retinal damage in albino rats. Use of opaque cages in cage racks under the recommended illumination level of 323 lux would substantially reduce the illumination level to which the animals are exposed (approximately 32-40 lux) and should be used to help prevent light-induced retinal damage. If albino rats are to be housed for extended periods of time it may be prudent to use levels of room illumination lower than those recommended since Weisse et al. (1974) have shown that the retinas of albino rats are extensively damaged by 2-3 years exposure to 32 lux.
In addition, it might be useful to consider the use of non-albino rats which have been shown to be less sensitive to light-induced retinal damage (Rapp and Williams, 1980).

The pigment epithelium plays a vital role in the normal maintenance of the retinal photoreceptors. The cells of the pigment epithelium are involved in the processes of photoreceptor outer segment renewal and visual pigment regeneration, provide a protective blood-retina barrier between the choriocapillaris and the photoreceptor cells, and provide metabolic and structural support to the outer retina. Experiments designed to determine the effects of light on the functioning of the albino pigment epithelium suggest that this particular retinal tissue is altered by light intensities well below those which produce measurable changes in the neural retina (CHAPTER 4). The changes observed in the functioning of the albino rat pigment epithelial cells, as determined from recordings of the c-wave component of the ERG and sodium azide-induced changes in the resting potential of the eye, suggest that light affects transport processes across this cell layer (CHAPTER 4). These changes, whether in the plasma membranes of the pigment epithelial cells or in the tight juctions between the cells, could potentially alter the susceptibility of the photoreceptor cells in the albino rat retina to light damage by compromising the ability of the pigment epithelium to support these cells. The apparent light sensitivity of the albino rat pigment epithelium may be exceptional since the c-wave component of the ERG, which may be used as a measure of pigment epithelial function, is present in albino varieties of both rabbit and guinea pig and does not appear to be affected by low intensities of light (Weidner, 1967). The present study (CHAPTER 4), suggests that the physiological changes in the pigment epithelium occur well in advance of those which occur in the photoreceptor cells in response to toxic light exposure.

The results of this experiment raise several interesting questions concerning the effects of light on the physical characteristics of the albino at pigment epithelium. First, are the light-induced changes in the functioning of the Sprague-Dawley albino rat pigment epithelium unique to this strain of albino rats? Second, what is the nature of the light-induced cellular change and where does it occur? Third, are the changes reversible? Answers to these questions will provide information needed to better define the effects of light on the pigment epithelium and its role in retinal light damage.

The photoreceptor cells are the first cells of the neural retina to show signs of damage following toxic light exposure (CHAPTER 3; Noell et al., 1976; O'Steen and Lytle, 1971; Shear et al., 1973). These cells possess a remarkable ability to renew their photosensory region, a process which continues throughout the life of the cells and is essential for its survival. Studies to determine whether the retina of the albino rat is capable of recovering from light damage (Kuwabara, 1970; Kuwabara and Funahashi, 1976; Noell et al., 1966; O'Steen and Lytle, 1971; Saavedra and Pellegrino de Iraldi, 1976; Wyse, 1980) have shown that the retina is indeed capable of regeneration and suggest that the immature retina possesses a greater capacity for regeneration than the mature retina.

The processes of renewal in mature photoreceptors are normally required to maintain the outer segments at a constant length. The present study shows that mature photoreceptor cells continue to renew their outer segments at approximately normal rates during exposure to light intensities which damage these cells (CHAPTER 5). These results and those of other studies which have investigated the regenerative capabilities of the mature retina following light damage suggest that the rate of renewal is relatively constant and inflexible in the mature albino rat retina (CHAPTER 5). One important question which remains to be answered is whether light intensities which damage the retina alter the relative amounts of lipid and protein synthesized by photoreceptor cells. Further studies on the amount and types of membrane components synthesized by photoreceptor cells exposed to damaging light will be needed before the effects of light damage on the renewal process can be fully understood.

In conclusion, the present study shows (1) that the retinas of albino rats raised under 6 lux (12:12 L:D) are damaged by small increases (1 log unit) in cyclic environmental illumination, (2) that these increases in illumination affect both the pigment epithelium and the photoreceptor cells and (3) that photoreceptor cells do not adjust the rate of outer segment renewal in response to this damaging light.

One of the major unanswered questions still remaining concerns the effect of previous light history on the susceptibility of the albino rat retina to light damage. A recent study conducted by Penn and Williams (1985) suggests that young photoreceptor cells develop to perform optimally under ambient light levels present during the maturation process. Based on their preliminary results, one would expect that the retinas of albino rats raised under a higher light intensity than that used in the present study might not be damaged by the same light intensities reported here. Would the retina be damaged by a 1.0-1.3 log unit increase over the intensity of the rearing light as suggested by the present study (CHAPTER 3)? Further work in this area will be needed to define the susceptibility of the albino rat retina to light damage.

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BIOGRAPHICAL SKETCH

I was born March 30, 1955, in Ft. Riley, Kansas, and attended primary school in Minnesota. I graduated from Mounds View High School, in Minnesota, with interests in biology and chemistry. I majored in psychology and biology at Gustavus Adolphus College, graduating magna cum laude, in 1977. I then enrolled in the psychobiology program at the Unversity of Pittsburgh to study with Dr. E. Bruce Goldstein with whom I obtained a M.Sc. degree in 1979. In 1979, I began work with Dr. Theodore W. Berger at the University of Pittsburgh. While studying at the University of Pittsburgh, I met and married my husband, Dr. Neil Rowland. My first son, Matthew Eric, was born in Pittsburgh in 1981. In 1981, I entered the Ph.D. program in neuroscience at the University of Florida and began work with Dr. William W. Dawson. My second son, Nilsson Stanley, was born in 1984.

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